

**DISPERSIONS OF
LAMELLAR LIQUID CRYSTALS
IN WATER AND WATER-OIL SYSTEMS**

Lisbeth E.M. Rydhag

 lund 1982

STOCKHOLM 1982

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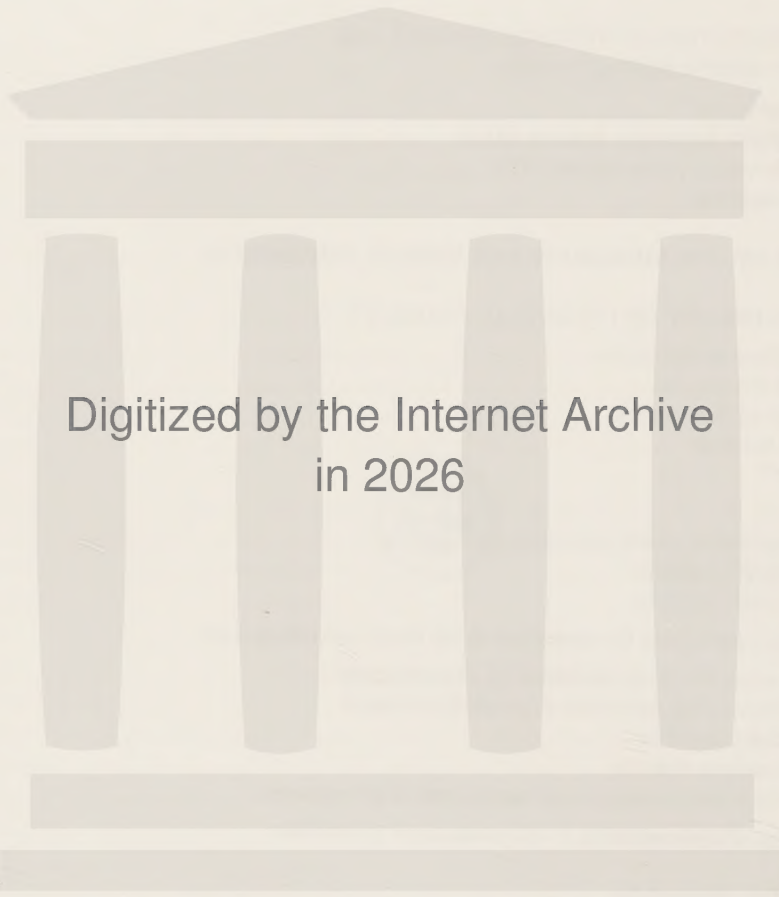
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INTRODUCTION

This investigation is concerned with the possibility to correlate the colloidal stability of dispersions of lamellar liquid crystals in water, (i.e. liposomes) and in water-oil systems, (i.e. emulsions) with the phase equilibria of binary surfactant/water and ternary surfactant/water/oil systems. The thesis summarizes studies of the stability of dispersions and emulsions containing surfactants which form lamellar liquid crystals in water or at water-oil interfaces. Results from the following papers are treated:

- I. Friberg, S. and Rydhag, L. The System: water/p-xylene/1-aminooctane/octanoic acid. II. The stability of emulsions in different regions. *Kolloid Z. u.Z. Polymere* 244:233-239. 1971.
- II. Rydhag, L. And Friberg, S. Phase equilibria of water/hydrocarbon/non-ionic emulsifiers. VI Intern. Kongress für grenzflächenaktiven Stoffe, Zürich, 1972.
- III. Rydhag, L. The importance of the phase behaviour of phospholipids on the emulsion stability. *Fette, Seifen, Anstrichmittel* 81, 168-173, 1979.
- IV. Rydhag, L. and Wilton, I. The function of phospholipids of soybean lecithin in emulsions. *J. Am. Oil Chem. Soc.* 58, 830-37, 1981.
- V. Rydhag, L. and Gabrán T. Phase equilibria in the system; Dimyristoylphosphatidylcholine/N-hexadecyl-N,N,N-trimethylammoniumbromide and water at 30°C. Swelling behaviour of the lamellar phase with different electrolyte solutions. Accepted for publication in *Chem. Phys. of Lipids*.
- VI. Rydhag, L. Stenius, P. and Ödberg, L. Phase equilibria and vesicle stability in dimyristoyl phosphatidylcholine/cetyltrimethylammoniumbromide systems. *J. Coll. Interface Sci.* 86:1, 1982.

The phase equilibria of the surfactants have been determined according to the methods developed by Ekwall and co-workers (1). These methods include the establishment of the one, two and three phase regions of the ternary system as well as the identification of the various liquid crystalline phases in the ternary system. The colloidal stability of vesicle dispersions has been investigated by measuring the flocculation rate at different electrolyte concentrations (2). The rate constants for the coagulation of vesicles have been calculated. For oil-in-water emulsions stabilized by surfactants which organize into lamellar liquid crystals, the emulsion stability has been investigated by various methods including determinations of oil separation in the presence of electrolyte, drop size distribution and electrophoretic mobility.

A brief presentation of the most common phases occurring in surfactant/water/oil systems is given below. The optimization of surfactants for the preparation of stable vesicles and oil-in-water emulsions by the knowledge of the phase equilibria of the components will be discussed in relation to existing methods used for the optimization of such systems. A brief introduction to the DLVO-theory for colloidal stability and to the kinetics of flocculation will be given before the discussion of possible stabilizing mechanisms of vesicle dispersions and o/w-emulsions. Some suggestions are made concerning possible mechanisms for the stabilizing effect of lamellar liquid crystals in o/w-emulsions and in vesicle systems.

LYOTROPIC LIQUID CRYSTALS IN BINARY AND TERNARY SYSTEMS OF SURFACTANTS IN WATER AND WATER-OIL

The dualistic properties of surfactant molecules, i.e. the fact that their molecular structure can be divided into a hydrophobic and a hydrophilic region, promote the self-association of surfactants into micelles and liquid crystals (3, 4). With respect to the nature of the hydrophilic part of the surfactant molecule certain classifications are used namely anionic, cationic and zwitterionic surfactants while emulsifiers, solubilizers, wetting agents etc are classified according to their function (5).

In very dilute aqueous solution the surfactants are monomeric or form aggregates containing a few molecules. When the critical micelle concentration (cmc) is reached spherical aggregates of 15-100 monomers called micelles appear. These micelles consist of surfactant molecules with their polar head groups oriented at the surface of the aggregate facing the surrounding water. By the formation of micelles the contact area between the hydrophobic part of the surfactant molecule and water is minimized. Micelles are highly dynamic systems. The life-time of a micelle of sodium dodecyl sulphate is approx. a milli-second (6). The micellization depends not only on the concentration and the temperature but also on the binding of gegenions to ionic surfactants. The lowest temperature at which micelles are formed, i.e. where the surfactant solubility reaches the cmc is called the Krafft point (7). The interior of the micelle consists of a hydrocarbon region which is very similar to liquid hydrocarbon (8, 9). Organic substances which are insoluble in water can be incorporated in the liquid interior of the micelles. This phenomenon is called solubilization (5). Completely hydrophobic substances such as hydrocarbons are solubilized in the interior of the micelles while amphiphiles with weakly polar groups may be solubilized at the surface or in the palisade layer of nonionic micelles (5).

When the concentration of the surfactant in a binary system is increased above the solubility region a lyotropic liquid crystal separates. The most common mesophases are the normal hexagonal phase and the lamellar phase (10) (Fig. 1 a and b).

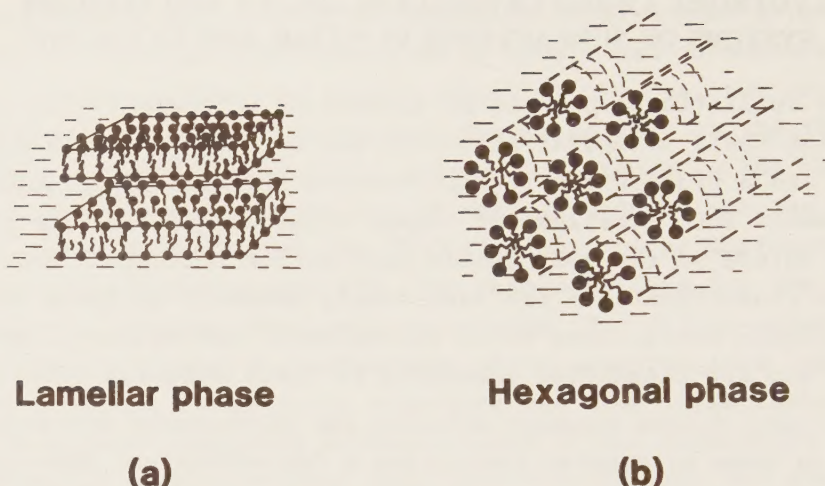


Figure 1. Lyotropic liquid crystals with lamellar (a) and hexagonal (b) packing.

When sufficient amounts of solubilize are added various crystalline phases may separate. The complicated phase equilibria of ternary systems of surfactant/water and a third component have been thoroughly investigated by Ekwall and co-workers (1, 11). Recent reviews dealing with the aggregation of surfactants in aqueous solutions have been published by Lindman and Wennerström (6, 9).

Liquid crystalline structures are formed when the solubility limit in the micellar solution is exceeded. The structure of the various mesophases have mainly been investigated by means of X-ray diffraction measurements (10, 12). Among the lyotropic liquid crystals the normal hexagonal, the reversed hexagonal and the lamellar phase are the most thoroughly characterized ones. The hexagonal phases are built up of infinitely long cylinders which are packed in a two-dimensional hexagonal lattice. In the normal hexagonal structure the surfactant aggregates are surrounded by water, in the reversed structure the hydrophobic part of the surfactants are located on the surface of the aggregates. The lamellar phase consists of alternating sheets of water and hydrocarbon chains of indefinite extension in two dimensions, separated by the polar groups of the surfactant. A variety of other phases have been proposed by many authors (12-16) but some

of them have been questioned with respect to their structure and/or existence (17-19). Measurements of monomer translational diffusion in lamellar and cubic phases have shown that the diffusion of monomer is of the same order of magnitude as in a monomeric solution (6, 20). The lateral diffusion coefficient for lipids in bilayer vesicles is approximately the same as that determined for multilamellar arrays (21).

The bilayer thickness of the surfactant spheres, cylinders or bilayers are mostly slightly shorter than twice the length of the extended hydrocarbon chains. The dimensions of the water regions may vary considerably between different phases or with the concentration of surfactant in a single phase.

CHARACTERIZATION OF O/W EMULSIFIERS AND VESICLE FORMING SURFACTANTS

Surfactants which are used as emulsifiers have been characterized by several methods such as film formation at air-water interfaces (22-24), hydrophilic lipophilic balance (HLB), (25-26), phase inversion temperature (PIT) (27-28) and by the thermodynamic phase equilibria of the surfactant/water/oil systems (24, 29).

Film formation

Schulman and co-workers studied the film formation of emulsifiers at the air-water interface by means of a Langmuir - Harkins balance. They found that when a water-insoluble film spread on a water solution of surfactant some of the substrate penetrated into the insoluble monolayer. Some of the surfactants which penetrated the monolayer were displaced when the film was compressed. In other cases a definite molecular complex was formed between the monolayer and the penetrating molecules, which could withstand greater pressures than did either of the components. If such complex formation should take place at the interface the resulting film should possess greater strength and resistance to rupture, hence the emulsion droplets would be more stable. From an extensive study of oil-in-water emulsions Schulman and co-workers concluded that the condition for maximum stability of o/w-emulsions are satisfied when the interfacial film is charged. In general it was also found that the same substances which form stable complexes at the air-water interface also stabilize emulsions best. The conclusion that can be drawn from the work of Schulman and co-workers is that considerable information may be obtained on the nature of the interfacial film in emulsions from studies of the air-water interface (22, 23). It has, however, been observed that the conditions of the emulsifier film at the air-water interface are not always applicable at the water-oil interface. Maximum stability of o/w-emulsions is in fact obtained over a large range of areas per surfactant molecule at the interface and not only at the tightest packing of molecules as indicated

from the film balance studies (30). It can be questioned whether Schulman *et al.* really obtained molecular complexes of compounds such as alkyl-sulphates and alcohols. According to an investigation by Friberg *et al.* (31, I) complex formation between the surfactant and the amphiphile compound does not account for an enhanced emulsion stability.

Film formation of long chain surface active lipids at air/water and oil/water interfaces in relation to their ability to stabilize emulsions has recently been discussed by Larsson (24). Below their chain melting temperature this type of emulsifiers may form crystalline gel phases that are of great importance for the emulsion stability at such temperatures. Long-chain surface active lipids (in this case 1-monoglycerides) form two- or three-dimensional phases, with excess water of either the liquid crystalline or gel type. It was shown that the molecular arrangements in the surface films can be correlated to the two- and three-dimensional phases of such amphiphiles in aqueous systems. Due to a stronger molecular association in the plane of the emulsifier film the crystalline gel state gives better emulsion stability compared to an emulsifier film of the liquid crystalline type. Larsson concludes that the monolayer technique, which provides information concerning the surface film phases occurring at certain surface pressures and temperatures, is a powerful tool for the choice of emulsifiers (24).

Hydrophilic-Lipophilic Balance (HLB)

In the HLB-method, the hydrophilic-lipophilic balance of the surfactant is characterized by an empirical number, the HLB number which is derived from the weight percent hydrophile in the molecule (25, 26). An HLB number is assigned to each surfactant and the efficiency of the surfactant in different applications is judged from an empirically known correlation between the number and the functioning in different types of systems. However, a number of factors which are not accounted for when calculating the HLB-numbers influence either the effective HLB of the emulsifier or the required HLB of the oil phases. Some factors that alter the HLB are; molecular configuration, concentration and location of emulsifier and dissolved substances in the water phase (30).

Phase Inversion Temperature (PIT)

The PIT has been suggested by Shinoda as a measure of HLB for nonionic emulsifiers which contain ethoxylated chains in their hydrophilic groups. The PIT-method may be used to optimize the choice of emulsifiers in the real emulsion system, i.e. PIT reflects the effects of sizes and types of hydrophilic and lipophilic groups of the surfactant and gives information of the effect of the concentration of emulsifier, the phase volume, the type of oil phase, the additives in both water and oil phases and the temperature (27, 28, 32-36). Emulsions stabilized with nonionic agents are often w/o-emulsions at high temperature and o/w-emulsions at low temperatures. This highlights the change of HLB of such nonionics with temperature. The PIT which is the temperature at which reversal from o/w to w/o-emulsions takes place, is considered to be the temperature at which the hydrophilic-lipophilic property of a surfactant is just balanced. Thus Shinoda suggests that the PIT may be designated as the "HLB-temperature" (27).

Strong interactions between the emulsifier and the oil phase results in a lower PIT-value while the PIT is close to the cloud point of the surfactant in a system with weak interactions between emulsifier and oil phase. The PIT increases with the size of the hydrophilic group of the surfactant which is in accordance with the rules of the HLB-system (25). PIT is depressed with the addition of electrolytes in the water phase. The energy required to emulsify the system can be minimized if the emulsification is performed at the PIT since at this temperature the interfacial tension reaches a minimum value (34, 36). Maximum stability of o/w emulsions is obtained at a temperature 15-20°C below the PIT (35). A simple method to determine PIT by means of differential thermal analysis has been described by Matsumoto and Sherman (37). Shinoda *et al.* have shown that the PIT-system may also be applicable to emulsifiers of ionic surfactant, co-surfactant, water and hydrocarbon (38).

Phase equilibria

The thermodynamic phase equilibria of emulsifiers in water or in both water and oil phase have been used by several groups to characterize the surface active properties of emulsifiers (24, 29, 39-41). Information about the solubility and liquid crystalline regions as well as data about two- and three-phase regions are available in such phase diagrams. In a recent review paper, Ekwall has summarized phase diagrams of several anionic alkyl soaps combined with weak amphiphiles or hydrocarbons (11). Several phase diagrams have also been determined for, e.g. alkyl amines, monoglycerides and ethoxylated nonionics. Friberg *et al.* have investigated the correlation between the phase equilibria in systems of water-oil-surfactant and the stability of emulsions formed in such systems (29, 42-44, I, II).

During the nineteen-sixties it was also clearly demonstrated by Larsson, among others, that the lamellar liquid crystals formed by lipids in equilibrium with very dilute aqueous solutions could be very easily dispersed in such solutions and would then form multi- or unilamellar particles, i.e. liposomes and vesicles (45).

STABILITY OF O/W-EMULSIONS AND VESICLE DISPERSIONS

An emulsion is according to its definition a dispersion of at least one liquid in another, i.e. from the thermodynamic point of view a two-phase system, the dispersed phase consisting of microscopic droplets in the size range of 10^2 to 10^5 nm. Hence coalescence of droplets is a thermodynamically spontaneous process whereas the opposite requires work and therefore does not occur spontaneously.

In order to prepare a stable emulsion with a persistent concentration of disperse phase it is essential to add a third component of a particular type, i.e. an emulsifying agent and emulsifier. Surfactants containing one or several hydrophobic groups within the same molecule are strongly adsorbed and oriented at an oil-water interface. Macromolecular emulsifiers, for example certain biopolymers and also synthetic polymers are strongly adsorbed at the oil-water interface. Each molecule is attached at many points at the interface and acts as a steric stabilizer. This property of emulsifiers gives very stable emulsions. Finely divided insoluble particles which stick to the interface by reason of surface tension forces can also be used as emulsifiers. The optimal emulsion stability is obtained when the solids are equally wetted by the oil and water phases (30). The stabilizing ability of some surfactants belonging to the first type of emulsifiers will be discussed further below.

The relationship between the interaction of polar lipids with water, their formation of lamellar liquid crystals and their action as emulsifiers in o/w-emulsions has been reviewed recently by Friberg and Larsson (46). They found that the most common liquid crystal in emulsifier/water systems is the lamellar one and it is this phase that is dispersible to form liposomes. It appears that the emulsifier/water interaction is the decisive factor in obtaining the packing in planar layers which is the prerequisite not only in obtaining a liquid crystalline phase but also in forming an interfacial layer of the emulsifier in an emulsion. The optimum emulsion stability which frequently is obtained if two emulsifiers are combined in certain ratios is not due to a specific complex formation between the two molecules as was suggested by Schulman and co-workers (22). Emulsion

stability rather is obtained for ratios between the two surfactants which are chosen in such a way that they give rise to liquid crystals in equilibrium with dilute aqueous solution. The nature of the hydrocarbon plays as important role for the effectiveness of an emulsifier blend as it does for a single emulsifier.

This appears to be closely correlated with the phase equilibria obtained in the four component system; oil/water/surfactant/co-surfactant. If the concentrations of the surfactants and the oil/water ratio can be chosen in such a way that the system at equilibrium will contain oil, water and lamellar liquid crystal the conditions are favourable for the formation of stable emulsions. These equilibria will be substantially shifted by substituting, e.g. an aliphatic hydrocarbon for an aromatic one of the same molecular weight (II). From the practical point of view, thus, it appears evident that the function of many emulsifiers may be quantitatively correlated with their ability to form lamellar liquid crystals together with the oil and water phases of the emulsion system.

As mentioned earlier some emulsifiers such as long chain surface active lipids may form several crystalline phases below their chain melting temperature, of these the crystalline gel state is of great importance for emulsion stability at temperatures below the chain melting temperatures, *cmf* of the emulsifiers. This mechanism of stabilization which has been studied by Krog and Larsson (24, 47) will not be further discussed here.

The successful preparation of easily dispersable and stable vesicles of a certain size and charge has been described by several authors (48, 49). A more detailed knowledge of the mechanism that stabilizes such dispersions is required, however, to predict their properties when used as models for biomembranes or as drug carriers. In many cases it is found that the o/w-emulsions which are formed in systems where liquid crystals occur may vary widely in their stability. A prediction of this variability requires a deeper understanding of the stabilization mechanism. It is obvious that many of the lipid mixtures used in the preparation of vesicles and liposomes are

very similar to those used as stabilizers for emulsions and whose function in the emulsions can be correlated with their formation of liquid crystals. Hence, it is very probable that the mechanisms that stabilize emulsions and vesicles, respectively, are very closely correlated.

In order to obtain a more complete experimental background for a quantitative theory of this type of stabilization, the phase equilibria of lipids and the stability of vesicles and emulsions formed in similar systems have been investigated (III, IV, V, VI).

THE DLVO-THEORY OF COLLOIDAL STABILITY

The Diffuse Double Layer

The electric double layer of an interface can be regarded as consisting of two regions; an inner region in which there may be specific adsorption and a diffuse region in which ions distribute under the influence of electrical forces from the surface and random thermal motion. A quantitative treatment of the diffuse part of the double layer was made in the model proposed by Gouy and Chapman. In this model i) the surface is assumed to be flat, of infinite extent and uniformly charged, ii) the ions in the diffuse part of the double layer are assumed to be point charges distributed according to the Boltzmann distribution, iii) the solvent is assumed to influence the double layer only through its dielectric constant, which is assumed to have the same value throughout the diffuse part (50, 51).

The inner part is separated from the diffuse layer by a plane (Stern plane) located at about a hydrated ion radius from the surface. The location of specifically adsorbed ions is in the Stern layer and ions located beyond the Stern plane form the diffuse part of the double layer. The double layer as a whole is electrically neutral (50, 51).

The DLVO Theory

A quantitative theory for the stability of hydrophobic colloids in terms of energy changes which take place when particles approach each other was developed by Derjaguin and Landau (52) and Verwey and Overbeek (53). The theory involves estimations of the van der Waals attraction energy and the energy of repulsion in terms of interparticle distances.

The double layer repulsive interaction, V_R results from the overlapping of the diffuse parts of the double layers. An approximate equation for spherical particles of radius a is

$$V_R = \frac{64\pi\epsilon_0 kT\gamma^2}{N_A \kappa} \exp(-\kappa D) \approx 2\pi\epsilon_0 \epsilon_r a \psi_d^2 \exp(-\kappa D) \text{ for low } \psi_d. \quad (1)$$

D is the shortest distance between spheres of radius a . (A list of symbols is given at the end of this chapter).

The attractive part of the van der Waals' interaction attractive forces, V_A results from the London dispersive attraction energy between the molecules in the two particles. For two identical spheres of radius a , with the shortest distance D between the spheres this force in vacuum is given by

$$V_A = \frac{-A_H \cdot a}{12D}, \quad (2)$$

where A_H is the Hamaker constant.

The Hamaker constant depends on the nature of the material of the particles, $A_H \approx 10^{-20} - 10^{-19}$ J. The presence of a liquid medium between the particles lowers the attraction energy. Approximately, the lowering can be accounted for by replacing A_H in eq. (2) with $A_{12} = (A_{11} - A_{22})^2$ where A_{11} is the Hamaker constant of the particles and A_{22} the Hamaker constant of the medium.

The sum of V_R and V_A represents the total interaction energy. With properly chosen electrolyte concentration and surface potential etc this function will show a repulsive energy maximum at some distance D_m between the two particles, Fig. 2. If the maximum is much greater than the kinetic energy of the particles the colloid is stable. Addition of electrolyte lowers the maximum. If enough electrolyte is added to reduce it to zero the colloid flocculates rapidly, i.e. flocculation is completely diffusion controlled, Fig. 3.

At high surface potential the theory predicts that the critical coagulation concentration, ccc depends on the counter ion valence, z , as follows:

$$ccc \propto \frac{1}{z^6},$$

and at low surface potential as follows:

$$ccc \propto \frac{1}{z^2}$$

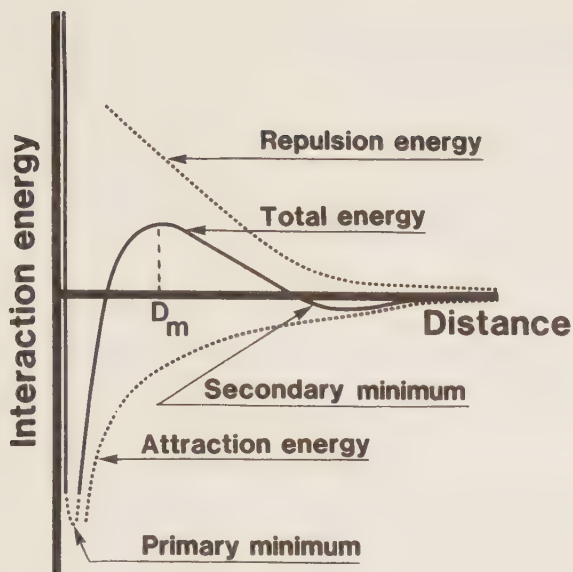


Figure 2. A total interaction energy curve with a maximum repulsion energy barrier at the distance D_m from the particle surface.

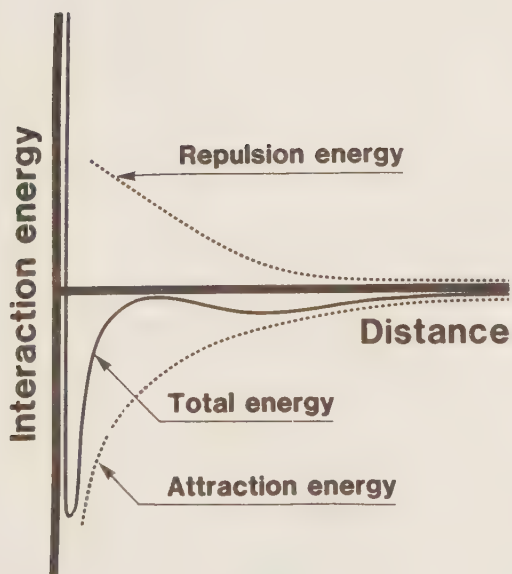


Figure 3. A total interaction energy curve without a repulsion energy barrier.

The relationship between ψ and the surface charge density is at low surface potentials (VI).

$$\log \psi = \frac{1}{3} \cdot \frac{\text{const}}{z^6} + \frac{4}{3} \log \sigma_d . \quad (3)$$

σ_d is the surface charge density, where the diffuse layer begins.

Kinetics of Flocculation

In the early stages of flocculation when only dimerization needs to be considered the rate at which particles aggregate is given by

$$-\frac{dN}{dt} = k_2 N^2 ,$$

where N is the number of particles per unit volume at time t and k_2 is the second order rate constant. Integrating and applying the boundary condition $N = N_0$ at $t = 0$ gives

$$\frac{1}{N} = k_2 t + \frac{1}{N_0} .$$

The rate of flocculation may be followed by measurements of the turbidity (50, 51). For a suspension containing N_0 particles per unit volume the turbidity for small particles ($d < \lambda/10$) is given by

$$\tau = B \cdot N_0 \cdot V_0^2 , \quad (4)$$

where

B = optical constant

N_0 = number of particles per unit volume

V_0 = volume per particle

For non-interacting particles whose diameter d is much smaller than the wavelength of the scattered light ($d < \lambda/10$) the turbidity is (50)

$$\tau = \frac{N_0 \cdot V_0^2 \cdot \rho^2 \cdot 32\pi^3 \cdot n_0^2}{3\lambda^4} \left(\frac{dn}{dc}\right)^2 . \quad (5)$$

Thus the optical constant B in eq. (4) is given by

$$B = \frac{32\pi^3 \cdot \rho^2 \cdot n_0^2}{3\lambda^4} \left(\frac{dn}{dc}\right)^2 . \quad (6)$$

In the first stage of coagulation it can be assumed that light is scattered only from monomers and dimers. Each formation of dimers reduces the particle number by one and creates a particle with $V = 2V_0$. From this and the second order rate law the following equation for the absorbance of the coagulating system as a function of time t may be derived (54).

$$A = \ln 10 \cdot B \cdot N_0 \cdot V_0^2 \left(\frac{1 + 2k_2 \cdot N_0 \cdot t}{1 + k_2 N_0 \cdot t} \right) \approx \ln 10 \cdot B \cdot N_0 \cdot V_0^2 (1 + 2k_2 \cdot N_0 \cdot t) \quad (7)$$

This approximation is only valid in the very initial state of the reaction (54). In spectrophotometers the recorded absorbance is usually defined as $A = \ln 10 \cdot \tau$ for non-adsorbing solutions (55). Thus the rate of change of absorbance with time is given by

$$\frac{dA}{dt} = k_2 (2B \cdot N_0^2 \cdot V_0^2) = k_2 \left[\frac{2B \cdot c_0^2}{\rho^2} \right]. \quad (8)$$

The experimental determination of dA/dt enables k to be obtained.

Combining Smoluchowski's equation for the rate constant of diffusion controlled disappearance of the particles (which is valid if there is no repulsive energy between the coagulating particles and there are no hydrodynamic forces that affect the coagulation except the friction between the particles and the solvent) (56) and the Stokes-Einstein's equation for the diffusion coefficient of spherical particles in laminar flow gives

$$k_0 = \frac{8 \cdot kT}{3\eta}. \quad (9)$$

In the presence of a potential energy barrier coagulation is slowed down and the rate constant, k_s , is decreased by a factor W :

$$k_s = \frac{8kT}{3 \cdot \eta \cdot W}. \quad (10)$$

Theoretical expressions for the correction factor, W , were derived by Fuchs (57) and by Reerink and Overbeek (58) which predict a linear relationship between $\log W$ and $\log$ electrolyte concentration at constant surface potential. At maximum repulsive energy and after insertion of all the known constants

for water at 25°C the expression derived by Reerink and Overbeek (58) becomes

$$\ln W = \text{const.} - 2.06 \cdot 10^9 \frac{\gamma_a^2}{z^2} \ln c_0. \quad (11)$$

The critical coagulation concentration (*ccc*) is the electrolyte concentration needed to obtain the diffusion controlled coagulation rate. Experimentally *ccc* is determined by plotting the logarithm of *W* (or the experimental rate constant) against the log electrolyte concentration. At *ccc* a clear break is observed (50, 51). The correction factor, *W* is also known as the stability ratio $W = k_0/k_1$, i.e. the coagulation rate of particles with a repulsive energy barrier is reduced by a factor *W* as compared to the diffusion controlled coagulation rate of these particles (50).

LIST OF SYMBOLS

The DLVO-theory

A_H = Hamaker constant

c_0 = bulk concentration of electrolyte

D = distance from surface

e_0 = charge of an electron

ϵ_0 = permittivity of vacuum

ϵ_r = relative dielectric constant of the solvent

k = Boltzmann constant

κ = inverse Debye length

N_A = Avogadro number

T = absolute temperature

z = counter ion valence

Ψ = surface potential

$$\gamma = \frac{\exp(z e_0 \Psi_0 / 2kT - 1)}{\exp(z e_0 \Psi_0 / 2kT + 1)}$$

Kinetics of flocculation

A = absorbance

B = optical constant

$(\frac{dn}{dc})$ = refractive index increment

k_2 = second order rate constant

n_0 = refractive index

N_0 = number of particles per unit volume

t = time

V_0 = volume per particle

RESULTS

Investigation of vesicle formation and stability

Phase equilibria. The conditions of vesicle formation and stability were studied in a model system composed of a swelling amphiphile, dimyristoylphosphatidyl choline, DMPC, and a cationic soap, cetyltrimethyl ammoniumbromide, CTAB. For this system the phase equilibria have been clarified (V). The results show that stable vesicles are formed only in a certain concentration region in the phase diagram, namely in the two-phase region in which the swollen lamellar phase and the dilute monomer solution of DMPC and CTAB (below the *cmc*) are in equilibrium. When the cmc of the soap (CTAB) in the water solution is exceeded the DMPC is solubilized in the micelles. This destabilizes the bilayer structure of DMPC. The region, where stable vesicles will form, is shown as the shadowed area in a schematic phase diagram of a swelling amphiphile and a surfactant forming micelles in water, Fig. 4.

In this model system the electrically neutral amphiphile, DMPC swells to approx. 40 wt-% of water. When small amounts of the positively charged soap CTAB is added to the DMPC (weight

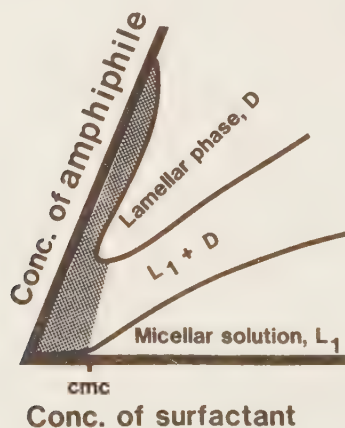


Figure 4. A schematic phase diagram of a water soluble surfactant and a swelling amphiphile in water. Stable vesicles are formed within the shadowed area.

ratio = CTAB/CTAB + DMPC = 0.01) the incorporation of water in the lamellar phase is dramatically enhanced.

A thermodynamic model for the description of the amphiphile association behaviour in ionic-amphiphile-water systems has been proposed by Jönsson *et al.* (59-60). The treatment is based on expressions for the different free energy contributions which in this model are i) a hydrophobic energy ii) a surface free energy of the aggregates iii) an electrostatic free energy iv) an entropy of mixing for the micellar aggregates and v) an energy that limits the aggregate size. From the free energy expression the chemical potentials of the different components are calculated and for each type of aggregate the optimal aggregate dimensions is determined. The stability regions of the different phases including two- and three phase regions have been determined for several soaps.

An estimation of the attraction and repulsion interaction energies was made according to this method (60). In the lamellar phase of DMPC and water (V) the interlamellar distance depends on the balance between the hydration repulsive energy and the attractive van der Waals energy. When the charged soap, CTAB, is introduced into the lamellar phase a repulsive electrostatic interaction energy arises which dominates at low surface charge densities. The calculations indicate that approx. 0.5 mole-% of the soap is required to obtain the enhanced swelling of the lamellar phase in water (61).

It should also be mentioned that the phase equilibria of this system are quite analogous to those of CTAB and a short chain alcohol, for example hexanol (62).

Vesicle stability. Concerning the nomenclature in this section, liposome includes both single bilayer and multilayer liposomes while vesicle is only used for liposomes with a single bilayer. Flocculation indicates a process in which aggregated vesicles remain as separate entities in the aggregate while in coagulation the vesicles coalesce and form one larger vesicle. The later process is often called fusion.

The kinetics of flocculation has been investigated for vesicles prepared in the two-phase region of the lamellar phase (D) and the monomer solution (L_1) in the ternary system of DMPC-CTAB-water at 30°C (V, VI). The results show that the

vesicles are electrostatically stabilized colloidal particles and the stabilities agree well with those predicted from the DLVO-theory. In summary, i) the stability with respect to the electrolyte concentration increases with an increase of the surface charge density of the vesicles ii) the linear relationship between the logarithm of ccc versus that of the surface charge density is obeyed (eq. 3) iii) the ccc decreases with increasing counter ion valence.

The ccc :s were experimentally determined for DMPC-vesicles with varying concentrations of CTAB in the presence of sodium chloride. The $\log W$ versus $\log$ electrolyte concentration curves are summarized in Fig. 5. From the theory a value for the rate constant, k_0 at diffusion controlled flocculation can be calculated from eq. (9). A value of $k_0 = 1.4 \cdot 10^{-11} \text{ cm}^3 \cdot \text{s}^{-1}$ is obtained for the diffusion controlled flocculation.

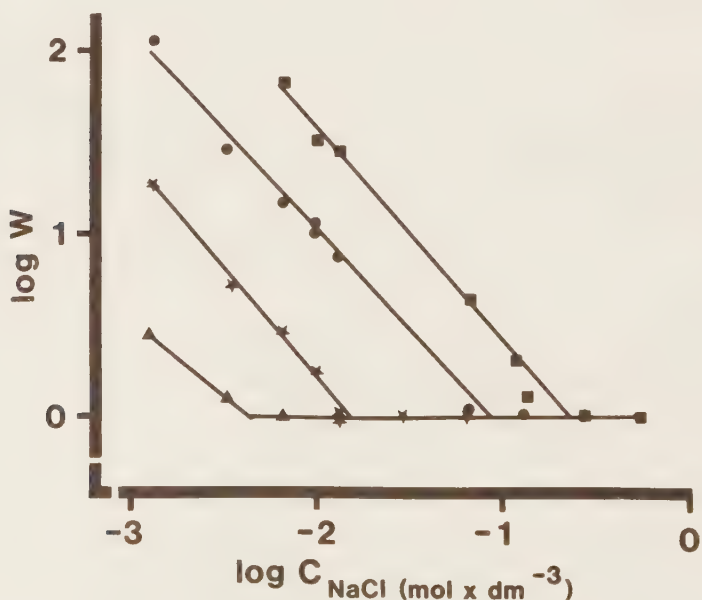


Figure 5. The critical coagulation concentration (ccc) for DMPC-CTAB-vesicles in water was determined for monovalent anions. The logarithm of the stability ratio, W , is given versus the logarithm of the electrolyte concentration. The CTAB concentration of the vesicles is

▲-▲	0.5 wt-% CTAB	●-●	5.0 wt-% CTAB
★-★	1.0 wt-% "	□-□	10.0 wt-% "

The experimentally obtained rate constants are, however, about three orders of magnitude lower than the values estimated from eq. (9), Table 1. Lansman *et al.* (63) have suggested that some ordering processes are necessary before the flocculated vesicles can coagulate (fuse). Nir and Bentz (64, 65) have pointed out that the equations of Smoluchowski or Fuchs (56, 57) should be corrected to take into account the hydrodynamic forces due to the finite rate of flow of the solvent in the region between two colliding particles. Spielman (66) has shown that the hydrodynamic correction factor modifies the diffusion coefficients at small distances of separation between the particles. This implies an increase in the factor W and, hence would further reduce the rate constant of aggregation of vesicles. Nir and Bentz (64, 65) have pointed out that this explains why Lansmann *et al.* (63) have obtained rate constants two orders of magnitude too low when compared to simple Smoluchowski coagulation for vesicles of phosphatidic acid and phosphatidyl serine. It should be noted that, in general, experimentally determined rate constants for the coagulation of any colloid are found to be considerably smaller than predicted by the Smoluchowski theory (51).

TABLE 1

Rate constants of vesicle coagulation

C_{CTAB} wt-%	Vesicle radius nm	$c_{\text{cc}}(\text{NaCl})$ mmole/dm ³	k_0 cm ³ · s ⁻¹
0.50	—	$4.0 \cdot 10^{-3}$	$8.1 \cdot 10^{-16}$
1.00	20	$1.3 \cdot 10^{-2}$	$8.1 \cdot 10^{-16}$
5.00	30	$8.9 \cdot 10^{-2}$	$2.0 \cdot 10^{-15}$
10.00	50	$5.0 \cdot 10^{-1}$	$2.3 \cdot 10^{-15}$

The coagulation rate was determined for 5 mM vesicle dispersions of DMPC-CTAB in water at 30°C. The optical constant, B was $5.2 \cdot 10^{18} \text{ cm}^{-4}$.

Emulsions stabilized by emulsifiers in the liquid crystalline state

In the first two papers (I and II) the emulsion stability was judged from determinations of emulsion type and phase separation. In the following papers III and IV the mean drop size and the drop size distributions were measured which gave the possibility to estimate the interfacial area of the dispersed phase. Determination of the electrophoretic mobility gave information about the sign of the net charge and surface potential of the droplets (III). Investigations are now in progress to find a suitable method to determine the particle growth rate of o/w-emulsions stabilized by phospholipids.

The emulsion stability was studied in a model system (water-p-xylene-1-aminooctane-octanoic acid) in which the emulsifier is changed from a liquid crystalline phase to an isotropic solution by altering the ratio of the two components constituting the emulsifier (I). Suitable oil to water ratios were chosen based on the phase equilibria of the model system, and the total emulsifier concentration varied. The results have given evidence of the stabilizing effect due to the presence of mesomorphic phases. This is in accordance with earlier results of Friberg *et al.* (29, 42, 44). The results also support the hypothesis that the stabilizing effect of the mesomorphic phase is independent of the means by which this phase is formed in the system. The suggestion that ordered layers of dimensions far exceeding the molecular size can be formed at the oil-water interface is supported and the multilayers of liquid crystals are suggested to act as a steric hindrance against the coalescence of flocculated emulsion droplets (I).

The correlation between the emulsion properties and the presence of liquid crystalline phases was investigated for two nonionic emulsifiers of commercial origin namely nonylphenyl polyglycol ether and dodecyl polyglycol ether (II). The mesomorphic phase was formed by changing the emulsifier concentration in the oil and water phases. From the knowledge of the phase equilibria, the concentration regions where the mesomorphic phases exist are known. The results demonstrate that the presence of a liquid crystalline phase in addition to the aqueous

and oil phases enhances the emulsion stability. It is shown that impurities in the emulsifier influence the phase behaviour and consequently the emulsion properties. In general the impure emulsifier gives rise to more stable emulsions at lower emulsifier concentrations. When an aliphatic hydrocarbon is replaced by an aromatic one the phase behaviour changes pronouncedly. The change is also accompanied by a corresponding alteration of the emulsion stability (II).

Oil-in-water emulsions stabilized by phospholipids

The swelling behaviour of emulsifier in water. A well-defined phosphatidyl choline (PC) swells to a lamellar liquid crystalline phase (D) above its chain-melting temperature (cmt). The lamellar phase contains at its maximum swelling about 40-50 wt-% of water. In the presence of minor amounts of electrically charged soaps or phospholipids the swelling of the lamellar phase in water is dramatically enhanced (67, III). Enhanced swelling of PC in water has also been observed in the presence of low concentrations of divalent cations such as Ca^{2+} , Mg^{2+} etc (68). When the ionic strength of the water phase increases, the swelling of the lamellar phase declines and at a certain ionic strength the repeat spacing of the lamellar phase is similar to that of the electrically neutral PC, i.e. approx. 60 Å. Addition of electrically neutral additives such as non-ionic surfactants does not change the maximal spacings of the lamellar phase (67, III). All phospholipids, independent of their origin swell in a similar way above their cmt . A similar swelling behaviour has also been observed for other surface active lipids such as monoglycerides (69) and dialkylamines (70).

The swelling behaviour of the lamellar phase indicates that the enhanced swelling in the presence of the charged groups depends on the electrostatic repulsion forces between the lamellae. Charged surfaces give rise to an electric double layer and when the ionic strength increases the diffuse double layer is compressed, i.e. the κ^{-1} decreases. The fact that

neutral additives such as fatty acids and nonionic surfactants do not change the repeat distance of the lamellar phase confirms this hypothesis (67, III). As mentioned earlier the phase boundaries in ternary systems may be predicted with great accuracy from calculations of the attraction and repulsion energies from the different compounds (59, 60).

Phase equilibria. Phosphatidyl choline (PC) from soybeans swells to a lamellar phase in water. The water rich part of that phase is in equilibrium with a very dilute monomeric solution of PC in water. (A solubility of approx. 10^{-10} M has been reported for PC from hens' eggs (71)) and a monomer solution of PC in soybean oil (SBO). In the presence of either an electrically charged soap or a phospholipid the lamellar phase swells to a greater extent in water (III).

Emulsion stability. The oil phase is more easily dispersed to small droplets and the stability with respect to coalescence increases when the emulsifier contains charged molecules. A certain amount of emulsifier is necessary to obtain stable o/w-emulsions. The critical emulsifier concentration corresponds to several layers of the emulsifier and the thickness of that layer is about 8-10 nm, i.e. four to five monolayers of emulsifier (III, IV). In the presence of electrolytes in the water phase very instable emulsions are formed. By increasing the fraction of charged lipids in the emulsifier the effect of electrolytes is depressed (IV).

Stabilizing mechanisms of surfactants in o/w systems. Kitchener and Musselwhite have discussed emulsion stability from a theoretical point of view (72). They conclude that the short range forces determine the structure of the interface whereas the long range forces decide whether the emulsion will flocculate or not. The application of the DLVO-theory to microscopic oil droplets stabilized by adsorbed emulsifying agents has also been discussed by Kitchener and Musselwhite (72). They have pointed out that the theory cannot be applied to the rate of coalescence of emulsion droplets since this process depends on displacing or disrupting an adsorbed film. The DLVO-theory may, however, describe the electrostatic contribution to stabilization of emulsions against flocculation. In emulsions - as

distinct from solid particles - several factors must be considered before the DLVO-theory may be applicable, namely the possibility of distortion of the droplets as they interact and the existence of a diffuse double layer within the droplets themselves. If the droplets are, to some degree, stabilized by double layer repulsion then close approach will tend to produce some flattening of the surfaces which will increase the potential energy barrier compared to the case of rigid spheres. The effect of an internal diffuse double layer in the oil phase has been discussed by Sherman (73). Some of the surface charge may then be neutralized by internal counterions and the surface potential is reduced. The repulsion between the droplets through interaction of their external double layers is otherwise unaffected by the existence of internal double layers.

In 1969 Friberg *et al.* (29) demonstrated that a pronounced stabilizing effect on o/w-emulsions was obtained when the emulsions were prepared in a concentration region where some of the water, emulsifier and oil formed a lamellar liquid crystalline phase at equilibrium. Friberg *et al.* (74) calculated the van der Waals attraction potential for two emulsion droplets covered with a multilayered liquid crystal and a monolayer of emulsifier. During the flocculation and coalescence process the van der Waals attraction potential draws the particles closer to each other. They found that the presence of the liquid crystalline phase caused a pronounced reduction of the available van der Waals energy for coalescence. The fact that the viscosity of the liquid crystalline phase is at least 100 times that of water indicates that the time of coalescence may increase by several orders of magnitude (75). Friberg *et al.* (74) have not considered the charge stabilization of o/w-emulsions in such systems probably because the emulsifiers used were either nonionic surfactants or electrically neutral zwitterions.

As mentioned earlier the present results from investigations concerning o/w-emulsions stabilized by phospholipids indicate that several monolayers of emulsifier are required to obtain stable o/w-emulsions (III, IV), which is in agreement with the results of Friberg *et al.* (74, 75). The experimental difficulties in the determination of the fraction of droplets, which

are less than 1 micron in size, may, however, lead to an underestimation of the total oil-water interface of the emulsion and thus an overestimation of the number of layers at the interface (IV). Another possibility is that the emulsifier is partly dispersed in both the water and oil phases (IV). Dispersible emulsifiers as such and in particular when combined with charged surfactants very easily disperse to form stable liposomes in the water phase (VI). The emulsifier may in the oil phase aggregate to reversed micelles (76). The adsorption of such emulsifiers to the oil-water interface seems, however, to occur more readily than from a water dispersion (IV).

It has been demonstrated that optimal stability is obtained for o/w-emulsions when the emulsifier or emulsifier-coemulsifier in oil and water form a lamellar liquid crystal in equilibrium with a dilute aqueous monomer solution and an oil solution of the emulsifier (74, II-IV). This is schematically illustrated in Fig. 6. It is important to notice that this occurs at a total concentration of emulsifier which is less than *cmc* (77). When the emulsifier forms a micellar solution the stability of the lamellar phase and thus, the emulsion stability, decreases (78). This is in good agreement with the observations on vesicle stability in the ternary system of DMPC-CTAB-water described earlier.

Tadros (79) who investigated xylene-in-water emulsions stabilized by CTAB and cetylalcohol observed a reduction of the interfacial tension when cetylalcohol was added to the system and simultaneously the adsorption of CTA^+ -ions to the oil-water interface increased. The author suggests that the increased adsorption of CTA^+ -ions may be due to a more dense packing of the ions as a result of the reduction of the repulsion between the headgroups when the alcohol adsorbs between the surfactant ions. Addition of the cetylalcohol also reduced the coalescence rates significantly. The enhanced stability is suggested to be due to the formation of a coherent strong interfacial film which acts as a "barrier" preventing coalescence. An emulsifier which is able to form a lamellar structure as illustrated in Fig. 6 might also be able to form a strong interfacial film as suggested by Tadros (79).

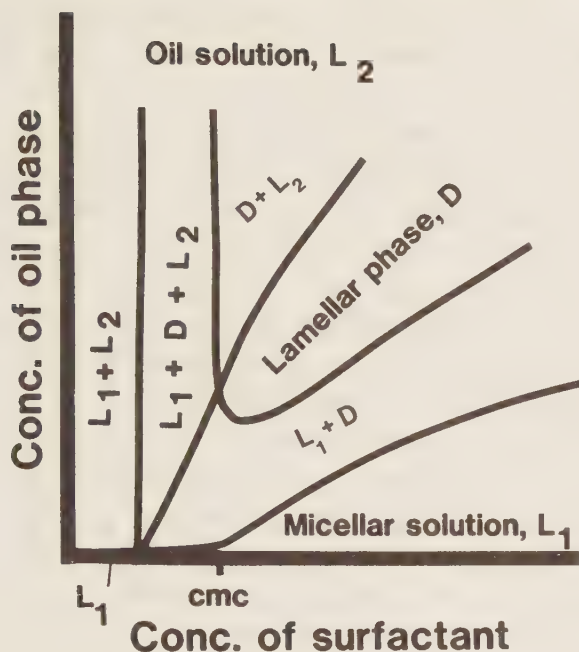


Figure 6. A schematic phase diagram of a surfactant-water-oil system at low surfactant and oil concentrations.

Stability of o/w-emulsions in the presence of electrolytes

Emulsion stability in the presence of electrolytes has been investigated for o/w-emulsions of soybean oil (SBO) in water. The emulsifier studied was phosphatidyl choline (PC) from soybeans combined with sodium stearate. The emulsions were prepared and characterized according to the descriptions in ref. (III). The oil to water phase ratio was 40:60 and the total emulsifier concentration varied between 1 and 4 wt-%. The freshly prepared emulsions were rapidly mixed with equal volumes of electrolyte solutions after which the separation of oil-phase was observed. The emulsions were centrifugated at low g-values (approx. 2500 x g) to make the process of oil separation faster. The separation of oil phase proves that the emulsified oil droplets have flocculated and then coalesced. The concentration of sodium stearate in the emulsifier has been given in mole-%

which is proportional to the surface charge density, σ . The sodium stearate is assumed to be equally distributed in the emulsifier PC.

The critical coagulation concentrations (ccc) for sodium chloride were determined for emulsions stabilized with an emulsifier containing between 2 to 20 mole-% of the charged component sodium stearate. The total amount of emulsifier was 1 wt-%, Fig. 7. From the relation between ccc and the surface charge density a linear relationship is predicted, eq. (3). This equation is valid provided that the particles have a low surface potential. The corresponding results for the coagulation with calcium chloride are shown in Fig. 8. The slope of the plots $k = 0.76$ and 0.61 respectively in Fig. 7 and 8 clearly deviates from the predicted value of $k = 1.33$. One interpretation of these results which imply a higher charge density than expected on the droplet surfaces would be that the sodium stearate preferentially orients at the oil/water interface

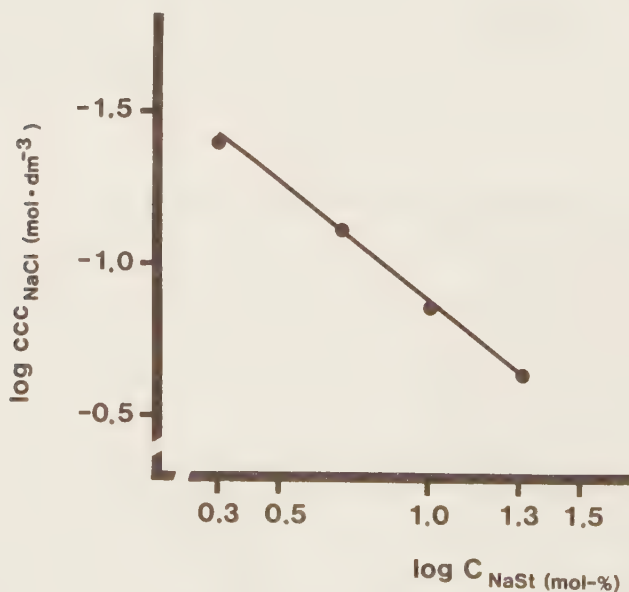


Figure 7. The critical coagulation concentration (ccc) for oil-in-water emulsions stabilized by soybean phosphatidyl choline and sodium stearate was determined for monovalent cations (Na^+). The total emulsifier concentration was 1 wt-%. The logarithm of ccc is given versus the logarithm of the charged component of the emulsifier.

enriched at the outermost layers. The values of k are approximately one half of the expected values. The relation between ccc and the counterion valence, z , is according to the DLVO-theory (50, 51) $ccc \propto 1/z^6$ for particles with a high surface potential and $ccc \propto 1/z^2$ for particles with a low surface potential. From the results in Fig. 7 and 8 a relation of $ccc \propto 1/z^7$ is obtained for emulsions with increasing concentrations of the charged component of the emulsifier, Table 2.

An investigation of the ccc for sodium chloride as a function of the emulsifier concentration shows an increase of the ccc with the amount of emulsifier, Fig. 9. At a constant surface charge density the ccc should not vary with the emulsifier concentration. These data support the assumption of a preferential orientation of the sodium stearate at the oil/water interface. A plot of ccc versus the total concentration of sodium stearate in these emulsions is shown in Fig. 10. It is obvious that this plot is almost identical to that of in-

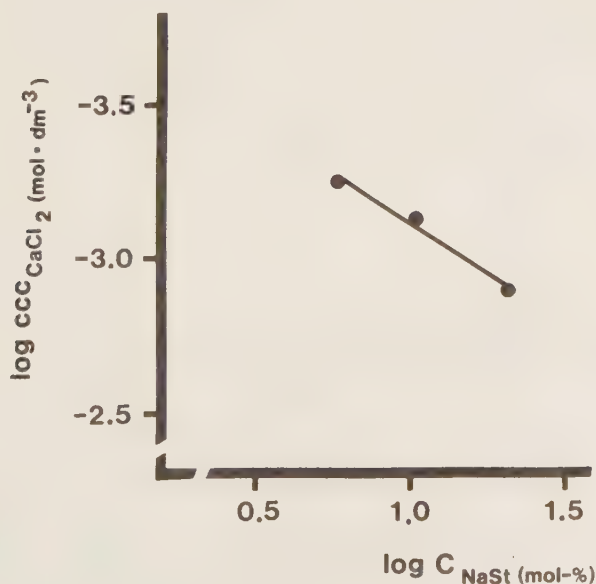


Figure 8. The critical coagulation concentration (ccc) for oil-in-water emulsions stabilized by soybean phosphatidyl choline and sodium stearate was determined for divalent cations (Ca^{2+}). The total emulsifier concentration was 1 wt-%. The logarithm of ccc is given versus the logarithm of the charged component of the emulsifier.

TABLE 2

The critical coagulation concentrations for mono- and divalent cations as determined for emulsion droplets with increasing surface charge densities

C NaSt. mole-%	ccc NaCl mole·dm ⁻³	ccc CaCl mole·dm ⁻³	ccc Na ⁺ / ccc Ca ²⁺
5	0.07	0.0006	1:0.0086
10	0.14	0.0008	1:0.0057
20	0.23	0.0013	1:0.0056

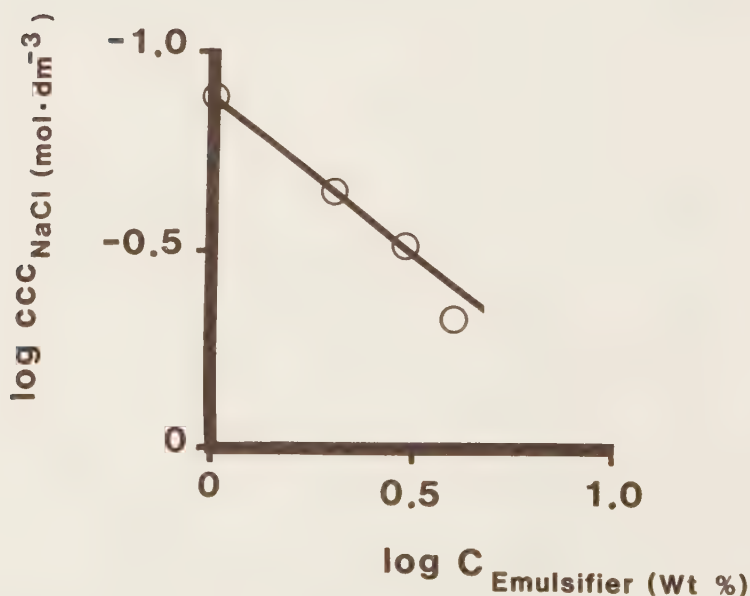


Figure 9. The critical coagulation concentration (ccc) for oil-in-water emulsions stabilized by soybean phosphatidyl choline and sodium stearate. The amount of sodium stearate was constant, i.e. 10 mole-% of the total emulsifier concentration. The logarithm of the ccc is given versus the logarithm of the emulsifier concentration.

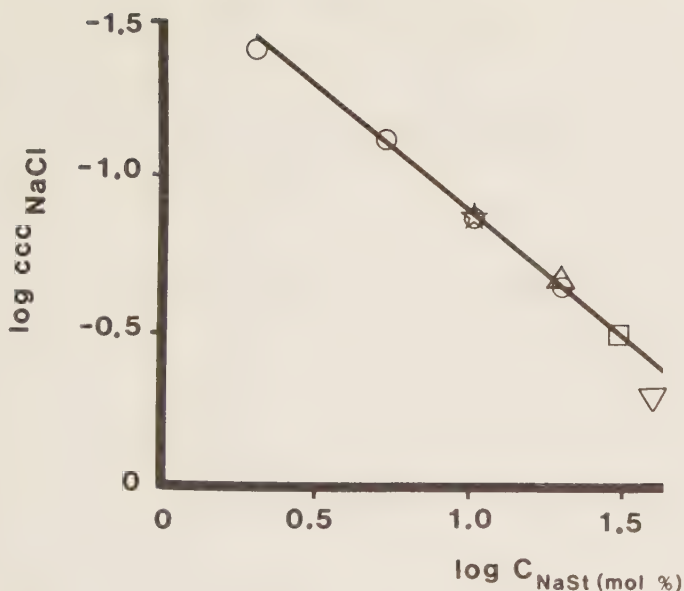


Figure 10. The critical coagulation concentration (*ccc*) for oil-in-water emulsions stabilized by soybean phosphatidyl choline and sodium stearate. The logarithm of the *ccc* is given versus the logarithm of the charged component of the emulsifier, -O-O-. The total emulsifier concentration was 1 wt-%. ☆, △, □ and ▽ indicate *ccc*:s for emulsions stabilized by 1, 2, 3 and 4 wt-% of emulsifier respectively.

creasing surface charge densities, Fig. 7. The stability of acoustically formed hydrocarbon in water emulsions prepared without stabilizers has recently been investigated by Reddy and Fogler (80). From analysis of the particle growth rate in the presence of electrolytes the authors conclude that the stability of these emulsions is due to a preferential adsorption of hydroxyl ions to the oil-water interface.

If the droplets are stabilized by charged molecules (sodium stearate) these give rise to a diffuse double layer. When two droplets approach each other the repulsion energy barrier prevents close contact. On addition of sufficient amounts of electrolyte the repulsion energy barrier disappears and the droplets may approach closely due to the van der Waals attraction forces (50, 51). The treatment of colloid stability is

different for dilute and concentrated dispersions (81). In dilute systems (i.e. vesicles) aggregation is a bimolecular process, thus the rate of coagulation (or coalescence) depends on the flocculation rate (30). In concentrated systems (i.e. emulsions) all particles interact simultaneously with several neighbours, i.e. the rate of coalescence does not depend on the rate of flocculation (30). If the particle radius becomes larger, both the repulsive, V_R and the attractive, V_A interaction forces increase, see eqs. (1) and (2), i.e. the maxima become higher so that the dispersion becomes more stable. When the particle radius grows the shallow minimum also becomes more pronounced. This may lead to secondary minimum coagulation, i.e. the particles aggregate into loose aggregates which easily can be redispersed (81). From the results it is obvious that the addition of electrolyte to the concentrated emulsion system, strongly accelerates also the rate of coalescence. This finally leads to the separation of the emulsified oil phase. Thus, these results are qualitatively consistent with the DLVO-theory but the rates of flocculation and coalescence have to be investigated in detail.

CONCLUDING REMARKS

Liposomes and in particular vesicles have been extensively studied as models for biomembranes and as drug carrier systems (49). To mimic natural membranes certain molecules such as immunoglobulines have been attached to the vesicle surface (82) and to increase the stability the interior of the vesicle bi-layer has been polymerized (83, 84). To make use of liposomes as drug carrier systems it is necessary to make stable and more concentrated preparations. This requires alternative mechanisms of stabilization such as the adsorption of protective colloids to the charged vesicle surfaces. In foods it is well-known that macromolecules such as proteins are effective stabilizers of oil in water emulsions (72).

Liposomes and vesicles are dispersions of lamellar liquid crystals in a monomer solution of the surfactant in water (45). When the vesicle forming surfactant associates to micelles in the water phase, the stability of the lamellae and thus the liposomes decreases (V). Knowledge of the phase equilibria makes it possible to optimize the choice of suitable surfactants for the preparation of stable liposomes. The liposomes are electrostatically stabilized colloidal particles, i.e. their stability with respect to electrolyte concentration and counterion valency may be predicted from the DLVO-theory (VI). The experimentally determined coagulation rates of vesicles are, however, several orders of magnitude lower than the predicted values. This suggests that there is some kind of repulsive energy barrier present which does not originate from the double layer repulsion. Small amounts of charged impurities, approx. 1 mole-% change both the swelling of an electrically neutral phospholipid and the colloidal stability of the liposomes. This may explain the variations in results obtained with phospholipids of different origin (V, VI).

The choice of surfactants functioning as emulsifiers might also be optimized by the knowledge of their association behaviour in oil-water systems (I-IV). When the emulsifier forms a lamellar structure which is in equilibrium with a monomeric solution of the emulsifier in water and a monomeric solution

in oil, oil-in-water emulsions of optimal stability are formed (II-IV). Calculations of the interfacial film thickness indicate that more than a monolayer of the emulsifier is required to produce stable emulsions (III, IV). This might depend on the difficulty to experimentally measure the fraction of droplets in the submicron range, which results in an underestimation of the total interfacial area (III-IV). The stability of oil-in-water emulsions containing partially charged emulsifiers may be predicted from the DLVO-theory. An increase of the emulsifier concentration is analogous to a rising concentration of the charged fraction of the emulsifier. This indicates that the charged surfactant is preferentially adsorbed to the oil-water interface. The most interesting of the available methods for optimization of emulsifiers is the PIT-method developed by Shinoda *et al.* (27, 28) for nonionic surfactants. It has recently been shown that the PIT for ionic surfactants depends on the amount of co-surfactant and the electrolyte concentration, as the PIT for nonionics depends on the temperature (38).

SUMMARY

The formation of stable liposomes as well as stable emulsifier films at an oil-water interface can be optimized with the aid of knowledge of the phase equilibria of these systems. When charged surfactants are involved they give rise to an electric double layer at the surface of the liposomes and oil droplets respectively. The stability of such dispersions is mainly governed by the interaction between the charge repulsion forces and the stability with respect to flocculation may thus be predicted from the DLVO-theory.

ACKNOWLEDGEMENTS

I wish to express my sincere thanks to Professor Stig Friberg for introducing me into this field of colloid science and for inspiring me to commence this work, and to Professor Per Stenius for providing me with stimulating working conditions and for advice and support during the work underlying this thesis.

I am grateful to Professor Kåre Larsson and Professor Björn Lindman for their never failing encouragement and guidance during this work.

I thank Dr Lars Ödberg for many valuable comments on this manuscript.

I am indebted to Mrs Inga Wilton for many valuable discussions and for her continuous interest. I also thank Mr Thomas Gabrán for excellent technical assistance.

I am grateful to Mrs Ulla-Britt Kronberg who skilfully typed this manuscript.

My sincere thanks to all my colleagues and co-workers during the years of fruitful cooperation and good fellowship.

Finally I thank my family for its understanding and encouragement which has greatly helped me to bring this work to its completion.

The present work was carried out at the Institute for Surface Chemistry, Stockholm, by grants from the Swedish Association for Surface Chemistry Research and from the Swedish Board for Technical Development.

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PAPER I

From the Swedish Institute for Surface Chemistry, Stockholm (Sweden)

The system: water-p-xylylene-l-aminooctane-octanoic acid

II: The stability of emulsions in different regions

By S. Friberg and L. Rydhag

With 6 figures

(Received August 3, 1970)

Introduction

The problems concerning the behaviour of emulsions have been the source of much interest during several decades, due to the technical importance of such systems. The stabilizing action of solid particles adsorbed to the surface is dependent on the hydrophilic-lipophilic balance of the surface (1-4), the size of the solid particles (5) and the electrostatic repulsion forces (6). Polymeric substances adsorbed to the surface also show efficient stabilizing action on emulsions. For this reason, extensive studies have been made of monomolecular layers of proteins and the relation between their rheological surface behaviour and their protective action as emulsifiers (7-10). New information on the protective action of the polymers appears to be available from the recent work by *Lyklema* (11) who has shown the variation in energy with a changed polymer configuration at the interface. The stabilizing action of monomeric emulsifiers has also been a source of interest, indicated by the numerous theories to their influence presented within a period of a few years (12-14).

For the long range forces, responsible for the protection against flocculation, the DLVO theory appears to give good results for O/W emulsions. The DLVO theory states that the interaction energy between two particles is determined by the sum of the *van der Waals* attraction and the electric double layer repulsion.

$$W = W_a + W_e. \quad [1]$$

W_a is generally evaluated by integration of the attraction between two molecules

$$W_m = -\frac{\beta}{d^6}.$$

Integration for two spheres gives after approximation (15)

$$W_a = \frac{A \cdot a}{12 S} \quad R \approx 2a \quad [2]$$

$$W_a = \frac{16 A \cdot a^6}{9 \cdot S^6} \quad R \gg 2a. \quad [3]$$

The same treatment for two flat plates gives

$$W_a = -\frac{A}{12\pi} \cdot \frac{1}{d^2}. \quad [4]$$

The influence of the intermediate material is regarded by a "dielectric factor" giving approximate formulae (16, 17).

The electric repulsion is calculated by using the *Poisson* equation to calculate the charge density and assuming the surface potential to be low enough to justify

$$\frac{e\Phi}{kT} \ll 1$$

which gives (16)

$$W_e \approx \frac{1}{2} D r \psi^2 \ln(1 + e^{-\kappa d}). \quad [5]$$

Secondary minima in the function (1) will be obtained under certain conditions, since the expression for the electric repulsion energy gives a maximum due to the squaring of the surface potential. These minima have also been calculated for emulsified droplets and it has been shown that the flocculation is a reversible process (17) giving doublet-singlet equilibrium, verified also experimentally (18).

This treatment is, however, valid only for the collection of droplets in the flocculation process. The stability preventing coalescence from the flocculated state is a matter of metastability of thin films against rupture. This stability is estimated to be dependent on four factors (19).

- The *Laplace* capillary pressure
- The electrical double layer repulsion
- The *van der Waals* attraction
- "Steric hindrance" of close-packed monolayers.

The "steric hindrance" is not well defined, but vaguely described as the influence of cohesion forces in a close-packed monolayer, preventing rupture after application of pressure. Monolayers stabilized by strong hydrogen bonds in the polar parts can indeed

withstand surface pressures of only a few dynes per cm less than the surface tension of water (20).

In our opinion, this "steric hindrance" is composed of several independent factors affecting the conditions of ordered layers of surface active substances at the water/oil phase boundary. The cohesion forces between the non-polar parts of the surface active substance are considered to be of less importance, except in their determination of the localisation of the substance to the phase boundary. The determining factors are probably found in the interaction of the polar parts, and the interaction of the water molecules with the polar and non-polar parts.

This is strikingly illustrated by the results of *Davies and Pugh* (13) showing a complete change of stability against coalescence of two emulsions of W/O type with nickel oleate as emulsifier, but with petrol ether as the oil phase in the less stable emulsion compared to benzene in the more stable. According to the authors no explanation could be given to this change of stability. It is, however, highly probable that the possible interaction forces between the benzene molecules and water (dipole-induced dipoles) or with the double bond in the oleate have a determining influence.

Friberg has earlier pointed to the formation of ordered systems of emulsifier, oil and water as a possible explanation of these phenomena (21). *Larsson* (22) had even earlier shown that stable "dispersions" could be formed from mesomorphic phases of monoglycerides and water after strong dispersive action in water. The mesomorphic phases of commercial emulsifiers reported by *Friberg* (23) were shown to have a decisive influence on the emulsion stability.

The results also gave evidence of the importance of interaction forces from the oil phase molecules to form ordered structures. Hexadecane and p-xylene gave pronounced differences in their capabilities to form ordered structures containing high amounts of water (23). In our opinion, this fact can give a new understanding of the problem encountered by *Davies and Pugh* (13). Since the structure of the oil molecules has such a determining effect on the regions of stability of the mesomorphic phases (23) it appears probable that the interaction at the interface can have a comparable influence on the stability against coalescence of emulsions.

These results gave a preliminary justification to assume that the spontaneous ordering

into mesomorphic phases of water, oil and emulsifier molecules at certain concentration ratios was a factor of determining importance when dealing with the stability against coalescence. The systems investigated up to now (24, 25) have, however, only permitted a variation of the oil/emulsifier ratio in order to obtain, or not to obtain, the conditions necessary for the formation of a mesomorphic phase. Regarding this, we found it desirable to investigate a system where not only the variation of oil-emulsifier ratio could be used to obtain the mesomorphic phase, but where also the nature of the emulsifier could be changed to give the same effect.

Friberg (25) has also suggested earlier that multilayers with structures similar to the mesomorphic phases could be formed at the interface due to the local increase of emulsifier concentration. This could also take place in cases where the total concentration ratio of emulsifier, oil and water did not permit the formation of mesomorphic phases. Before investigating the present system no experimental evidence of this has been reached.

The system of p-xylene-water-l-amino-octane-octanoic acid (26) was considered to be suitable for such an investigation. The amine forms a mesomorphic phase in connection with water. This mesomorphic phase can solubilize p-xylene and also the acid. The acid and the amine form a salt in the mesomorphic phase. The stability of the dimensions of the latter are not influenced by the addition of the acid to equimolarity with the amine. Additions in excess will change the phase into a solution.

Regarding this, the system had the properties required for the investigation, since the mesomorphic phase could be formed when keeping the water-oil-emulsifier ratio constant but varying the composition of the latter. The system has been used to determine the influence of the ordering forces on the emulsion stability giving rise to mesomorphic phases. The results have also given evidence of the formation of ordered structures at the interface when the total concentration ratios have not allowed the formation of mesomorphic phases.

Results

Fig. 1 shows the composition of the emulsions, and fig. 2 contains photographs of these after preparation and storage for five days. The composition of the two phases is marked on the phase diagrams in the figure.

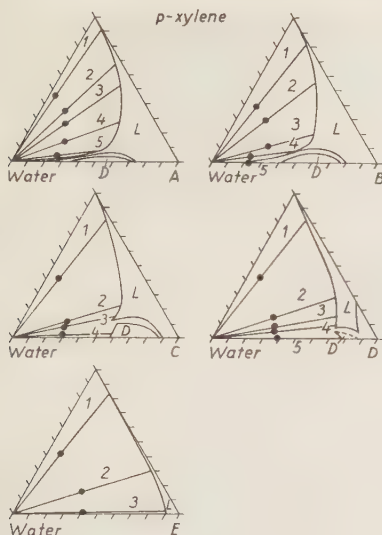


Fig. 1. Total composition of the emulsions in this investigation.

Emulsifier composition	A	B	C	D	E
Amine/acid weight %	100/0	90/10	66/34	40/60	25/75

In systems B-D, the last emulsion in every series contains a mesomorphic phase of the lamellar type. The presence of this phase gives emulsions which are highly stabilized compared to the others. Systems where the emulsifier composition prevents the formation of a mesomorphic phase, (A and E) give no emulsions of comparable stability.

The difference in the structure of the emulsions is shown by fig. 3, presenting the five emulsions of series B after storage for one hour, accompanied by microphotos of the emulsions which had partially separated into an upper W/O and a lower O/W emulsion. Emulsion No. 5 shows the presence of the liquid crystalline phase containing dispersed water. The photograph of this emulsion has been taken in polarized light, and shows ordered layers $\sim 1 \mu\text{m}$ around the included droplets.

Emulsions Nos. 1 and 2 are similar, which is also the case for Nos. 3 and 4. The first two show about the same stability for both the O/W and the W/O emulsion. Emulsions Nos. 3 and 4 have a considerably more stable O/W emulsion. It is notable how the W/O emulsion upper layer No. 1 differs from the corresponding No. 3 emulsion in having no flocculation and considerably smaller droplets. Emulsion No. 3 is flocculated to a very

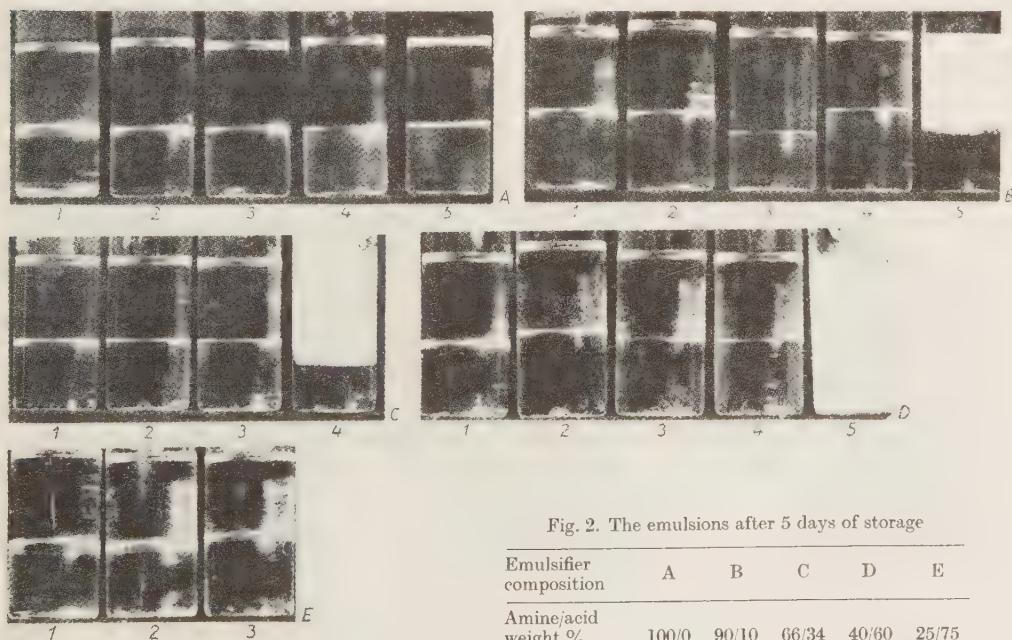


Fig. 2. The emulsions after 5 days of storage

Emulsifier composition	A	B	C	D	E
Amine/acid weight %	100/0	90/10	66/34	40/60	25/75

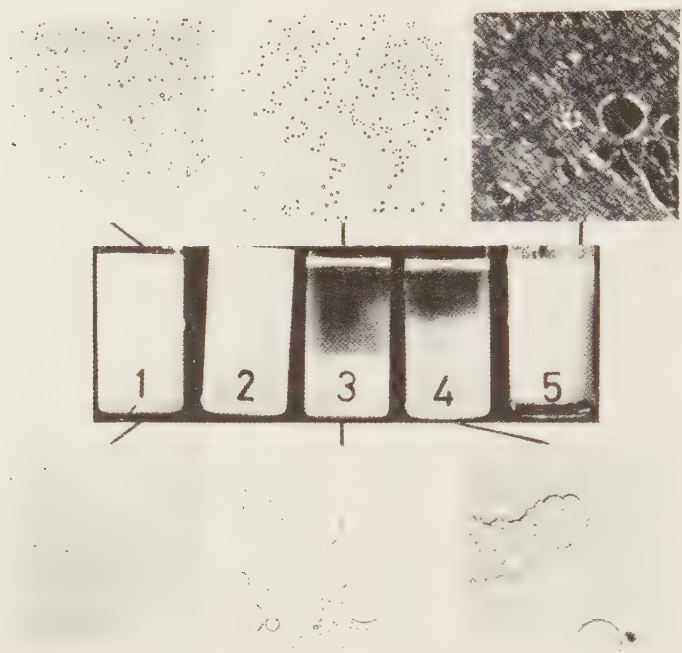


Fig. 3. The emulsions in series B after 5 min storage with microphotographs of the layers. Magnification $\times 300$

Emulsifier composition. B, 90/10 weight%					
Ratio emulsifier	1	2	3	4	5
p-xylene	0.2	0.5	3.1	6.3	44.0

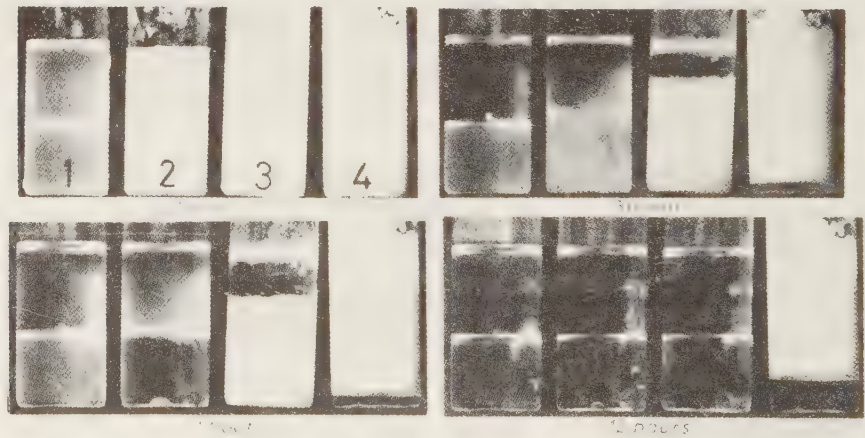


Fig. 4. The emulsions in series C after different times of storage

Emulsifier composition. C, 66/34 weight%				
Ratio emulsifier	1	2	3	4
p-xylene	0.2	2.5	3.9	15.0

high degree. These flocs will sediment considerably faster into the aqueous phase.

The lower parts of the corresponding O/W emulsions are considerably more stable in Nos. 3 and 4 than in Nos. 1 and 2. The reason is that emulsion Nos. 3 and 4 can tolerate intensive flocculation without coalescence. Emulsion No. 1 shows no flocculation at all, but the pronounced variation in size of the droplets points to coalescence.

This increasing instability of the (upper) W/O emulsion and the corresponding increase of the lower O/W emulsion is more pronounced in series C (fig. 4). The O/W emulsion No. 3 is considerably more stable than the others up to a storage time of 1 h. After 12 h, however, only the emulsion

containing the mesomorphic phase remains, the others, including No. 3, are completely separated. The microphotographs in fig. 5 show no flocculation in emulsion No. 1, but a wide size distribution pointing to coalescence having taken place. Emulsion No. 3, on the contrary, is completely flocculated; the continuous phase of the emulsion consists entirely of thin lamellas surrounding the dispersed phase. The photograph taken in polarized light shows the anisotropic nature of these parts. Emulsion No. 4 consists of water entrapped in the liquid crystalline phase.

The stability of the emulsions containing the mesomorphic phase is dependent on the composition of this phase. Fig. 6 shows the

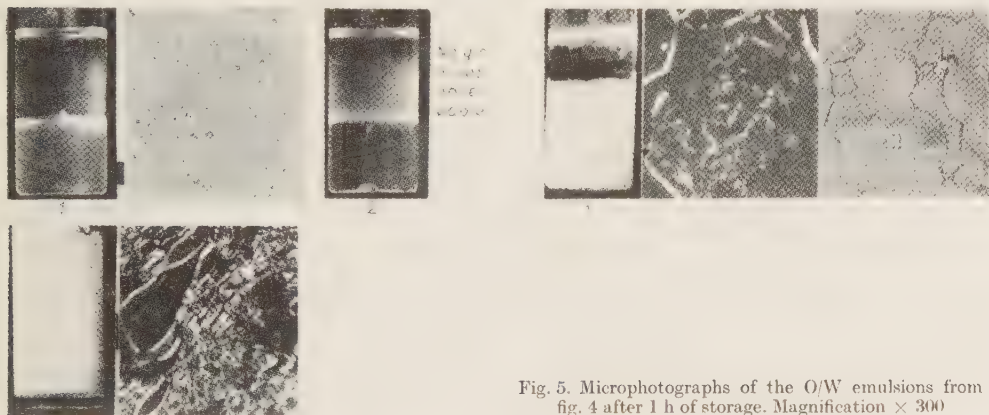


Fig. 5. Microphotographs of the O/W emulsions from fig. 4 after 1 h of storage. Magnification $\times 300$

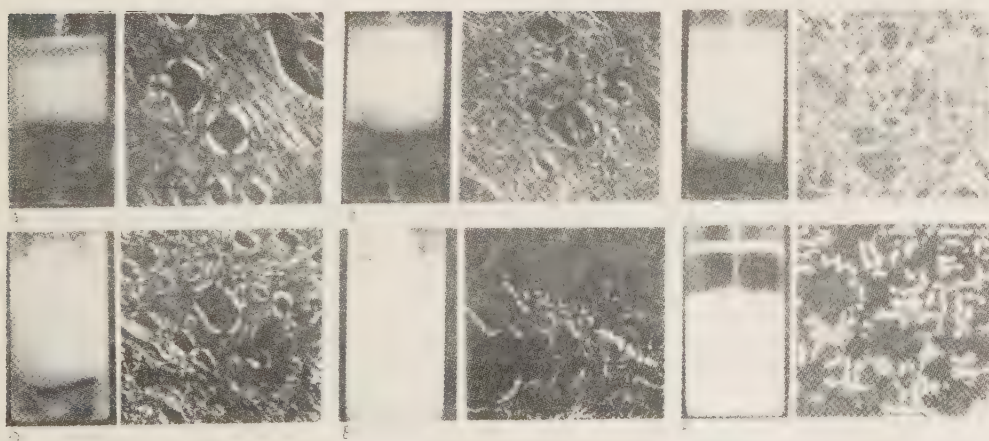


Fig. 6. Appearance and microphotographs of emulsions containing liquid crystalline phases after storage for a fortnight. Magnification $\times 300$

presence of these emulsions, where the ratio of octanoic acid: 1-amino-octane increases. Maximum stability is reached when the molar ratio of the two compounds is about 1:1. Less acid causes separation of a heavier liquid phase, and acid in excess causes the exclusion of a lighter phase. This phase does not contain p-xylene, but only water and amine.

The microphotographs taken in polarized light give evidence of the changed lamellar structure of the system, when the excess amine is finished in passing from D to E. The last "emulsion" consists entirely of thin lamellas surrounding the isotropic aqueous phase. The lamellas in system F appear to be less strong than in system E; they are of more uniform length and the thin network gives a more regular impression.

Discussion

The results have given evidence of the stabilizing effect due to the presence of mesomorphic phases. This is in accordance with earlier results of *Friberg et al.* (21, 24, 25). In earlier investigations, the mesomorphic phase was formed by means of changing the emulsifier concentration compared to the two liquid phases. This has also been done in the present investigation; emulsions with ascending numbers within each series contain increased amounts of emulsifier, and when the mesomorphic phase was formed the stability of the emulsion was suddenly increased. But the system used in the present investigation has also permitted the use of constant ratios of the three components, but with variations in the properties of the emulsifier by substitution of the amine by the acid. This does not change the packing properties in the mesomorphic phase formed by water and emulsifier as shown by the first article in this series (26). Excess acid will, however, give an isotropic solution instead of the mesomorphic phase, and no mesomorphic phase can occur in the emulsion. The same is true when the pure amine is the emulsifier. The mesomorphic phase is "masked" by the solution in this case (fig. 1). When the mesomorphic phase does not exist for these reasons, no emulsions of comparable stability can be prepared.

The results have supported the hypothesis that the stabilizing effect of the mesomorphic phase is independent of the means by which this phase is formed in the system.

The results have also supported an earlier suggestion that ordered layers of dimensions far exceeding the molecular size can be formed at the oil/water interface. Fig. 5 shows microphotographs illustrating such layers formed in a comparatively stable emulsion. This emulsion contains no mesomorphic phase. Fig. 4 gives evidence of a complete separation into two liquid phases after storage for 12 h. A possible explanation is that the ordering influence of the anisotropic conditions at the interface will supply the small amounts of energy necessary for the formation of an ordered structure.

The reason for the maximum stability of water dispersed into the liquid crystalline phase with an amine: acid ratio of about 1:1 cannot be given. The resistance to lateral movement will be at a maximum in this case, a fact that will increase the stability of the emulsion. On the other hand, the stabilizing effect of the electrical double layer will probably also reach a maximum at this composition.

Observing the system in fig. 6 gives the general impression that the lateral forces possibly have a more dominant influence in this case.

With these results, together with our earlier research in mind, we suggest that the "steric factor", responsible for the stability of ultra thin films, should not only be considered as due to the packing conditions of the hydrocarbon chains in monolayers, but that the total interaction pattern of oil-water-emulsifier should be observed. In some cases (21, 23) all three components appear to play equal roles in the formation of the ordered structures.

Acknowledgement

The investigations have been supported by the Swedish Board for Technical Development.

Summary

The influence of the presence of a "neat soap" mesomorphic phase on the stability of emulsions is shown by investigations on a model system of water, p-xylene, octanoic acid and 1-amino-octane. The mesomorphic phase could be formed in the system by increasing the emulsifier to oil ratio or by making the amine: acid ratio greater than above. In both cases, the stability of the emulsion increased suddenly when the system was changed in such a manner so that the mesomorphic phase was formed.

The investigations have also shown the presence of optically anisotropic layers in the region of the oil phase adjacent to the interfaces for stable emulsions, where no mesomorphic phase can be formed at equilibrium. This is considered to be due to the ordering influence of the interface, adding the small energy necessary to orientate the molecules.

Maximum stabilizing power of the mesomorphic phase is also found to be at a maximum when the amino: acid ratio is about 1:1.

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Authors' address:

Prof. Dr. Stig Friberg and Dr. L. Rydhag
The Swedish Institute for Surface Chemistry
Drottning Kristinas Väg 47, Stockholm (Schweden)

PAPER II

Sonderdruck aus

Chemie, physikalische Chemie und Anwendungstechnik der grenzflächenaktiven Stoffe

Berichte vom VI. Internationalen Kongreß
für grenzflächenaktive Stoffe
Zürich, vom 11. bis 15. September 1972

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Phase Equilibria of Water Hydrocarbons/Nonionic Emulsifiers

L. Rydhag and S. Friberg

The Swedish Institute for Surface Chemistry, Stockholm, Sweden

Résumé

L'influence de différents hydrocarbures sur l'équilibre des phases dans des systèmes eau/hydrocarbure/émulgateur non-ionique a été étudiée. La présence d'une phase liquide cristalline dans le système à des conditions d'équilibre augmente la stabilité des émulsions préparées avec les constituants respectifs. Les hydrocarbures aliphatiques et aromatiques provoquent des différences prononcées d'équilibres de phases et de stabilité d'émulsions.

Zusammenfassung

Der Einfluss verschiedener Kohlenwasserstoffe auf die Phasengleichgewichte in Systemen Wasser/Kohlenwasserstoff/nicht-ionischer Emulgator wurde untersucht. Die Gegenwart einer flüssigen kristallinen Phase unter Gleichgewichtsbedingungen im System bewirkt höhere Stabilität der Emulsionen aus den betreffenden Komponenten. Aliphatische und aromatische Kohlenwasserstoffe zeigen ausgeprägte Differenzen in den Phasengleichgewichten und in der Emulsionsstabilität.

Summary

The influence of different hydrocarbons on the phase equilibria in systems of water, hydrocarbons and nonionic emulsifiers was investigated. The presence of a liquid crystalline phase in the system at equilibrium conditions gave rise to enhanced stability of emulsions, which were prepared with corresponding compositions. Aliphatic and aromatic hydrocarbons gave rise to pronounced differences in the phase equilibria and accompanying differences in emulsion stability.

The possibility that emulsions may contain liquid crystalline phases has been considered several times in the last fifteen years /1,2/ and investigations have been made on nonequilibrium systems giving rise to expressions such as "selfbodying action" of some additives /3/. The first comparison of phase equilibria in three-component systems and the corresponding properties of emulsions were made at the Swedish Institute for Surface Chemistry /4/ using the techniques which had been developed by Ekwall and his co-workers /5,6/.

Earlier work by us /7,8/ was aimed at the observation of the change in emulsion stability when the liquid crystalline phase was intro-

duced by the increase of emulsifier concentration and by changing the oil/emulsifier ratio. In the present contribution we report results which describe the emulsion behaviour, when the three-phase area water, oil and liquid crystalline phase is changed into a two-phase region containing oil and a liquid crystalline phase. Changes in phase equilibria are also used to explain the influence of emulsifier impurities and the variation in the nature of the hydrocarbon in the oil phase.

Experimental

Materials

Nonionic emulsifiers of commercial origin, nonylphenyl polyethylene glycol ether, Emu-09 and dodecyl polyethylene glycol ether, Em-05, were supplied by Modokemi AB, Sweden. The nonionic emulsifiers were carefully purified of water soluble impurities and the emulsifier Em-05 was distilled under reduced pressure to remove organic impurities such as alcohols.

Hexadecane, puriss, Fluka AG Switzerland

p-Xylene, puriss, Fluka AG Switzerland

Paraffinic oil. Esso Primol 355, Svenska Esso, Sweden

All water was twice distilled.

Procedure

Phase equilibria

The samples for investigation of phase equilibria in the three-component systems were weighed directly into glass ampoules which were sealed by fusion. The samples were heated to homogeneity, slowly cooled to 20 °C under agitation, and allowed to stand at this temperature. The different phases were separated in an ultra-centrifuge and identified under a polarizing microscope or by X-ray methods according to previous work on phase equilibria in ternary systems /5,6/.

Emulsions

The different components were weighed into ampoules which were sealed by fusion, heated to homogeneity, cooled to 20 °C and finally

treated in an ultrasonic device for 1 min. One series in the three-component system, purified Em-05, hexadecane and water, was investigated using air filled, nitrogen filled and evacuated ampoules. No differences in stability or drop-size distribution were found, so air filled ampoules were used throughout the investigations. The type of emulsion was determined by the visual observations of the spread of oil- and water soluble dyes (Sudan III and Brilliant Blue F.C.F.) and controlled by diluting the emulsions with the continuous phase. The emulsion stability was determined by judging the separation rates from photographs.

Results

Phase equilibria for the system water/nonionic emulsifier (purified Em-05) hexadecane are presented in Fig. 1. The system (Fig. 1) consisted of a continuous liquid phase covering the emulsifier/hexade-

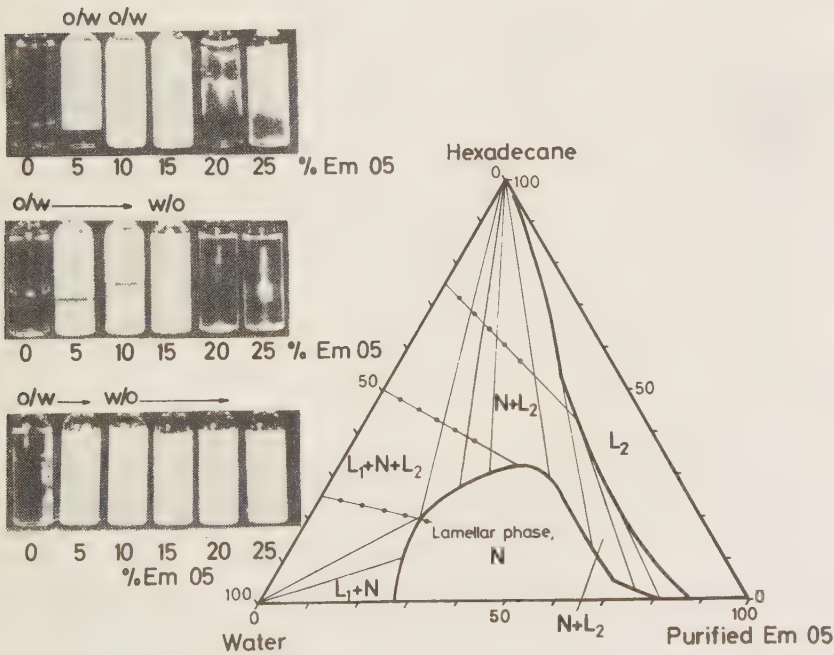


Fig. 1
Phase equilibria and emulsion stability for the system Purified Em-05/
Hexadecane/Water

cane axis and a liquid crystalline phase, a neat phase which solubilized between 20 - 30 % hexadecane. Phase equilibria for the system water/emulsifier, Em-05, still containing the organic impurities and with paraffinic oil instead of hexadecane is shown in Fig. 2. In this system a corresponding liquid phase was found, while the solubilizing capacity of the neat phase had been reduced.

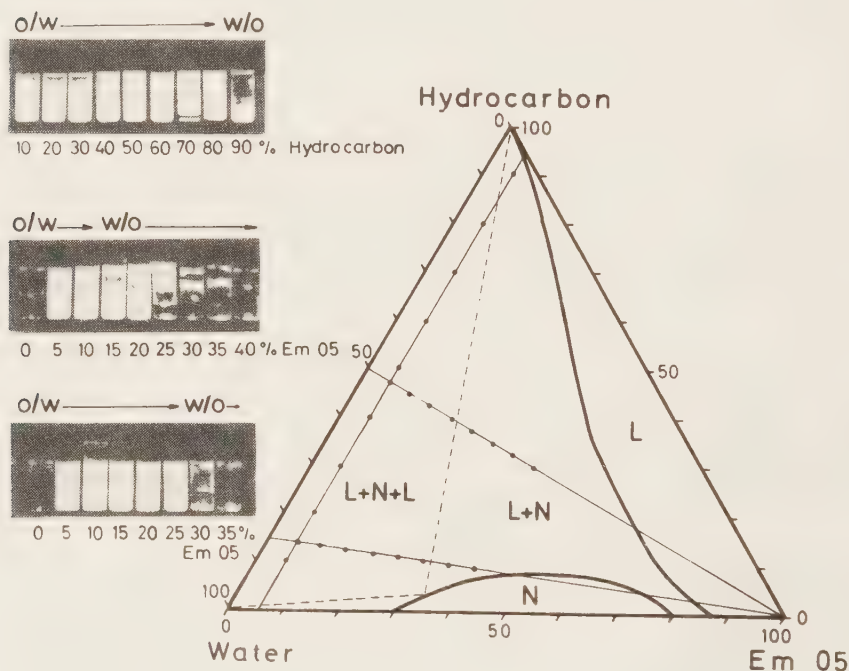


Fig. 2
Phase equilibria and emulsion stability for the system Em-05/Paraffinic oil/Water

Emulsions were prepared with different oil/water ratios and with emulsifier concentrations ranging from 5 to 25 or 35 per cent. In the system shown in Fig. 2 emulsions were also prepared with a constant amount of emulsifier (5 %) and different water/hydrocarbon ratios. Photographs of the emulsions, taken after 3 months, are shown in Figs. 1 and 2. The results all indicated a high stability in the three-phase regions where water phase, oil phase and a liquid crystalline neat phase were present. At higher emulsifier concentrations in the two-phase regions containing the oil phase and

the neat phase the emulsions were separated into two phases. Stable emulsions could be prepared in the three-phase regions with only 5% of emulsifier over a wide water/hydrocarbon ratio in the system water-Em-05-paraffinic oil (Fig. 2). The unstable emulsion with 90 % hydrocarbon was actually in the two-phase region, containing only the oil and the neat phase.

Phase equilibria for the systems water-nonionic emulsifier, type nonylphenyl polyethylene glycol ether and different hydrocarbons are presented in Figs. 3 and 4. The phase region in the system water/nonionic emulsifier (Emu-09)/hydrocarbons changed when p-xylene was replaced by hexadecane. The area of the neat phase was reduced and that of the middle phase increased. The liquid phase L_2 was reduced to a small area where the emulsifier dissolved small amounts of hydrocarbon and the aqueous solution was divided into three parts, one micellar solution and two isotropic liquid crystalline phases /5/.

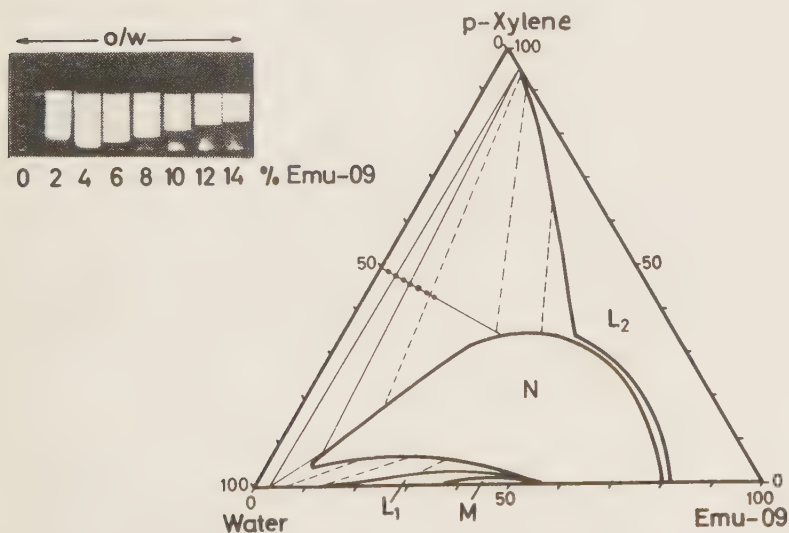


Fig. 3
Phase equilibria and emulsion stability for the system Emu-09/
p-Xylene/Water

Emulsions were prepared with equal amounts of water and hydrocarbon but with different concentrations of emulsifier. When passing from

the two-phase region (water/oil) into the three-phase region also containing the neat phase in the p-xylene system (Fig. 3), emulsion stability was increased. When the emulsifier concentration was further increased the aqueous phase disappeared and the stability of the emulsions was gradually reduced in spite of a reduced drop size. In the system with hexadecane (Fig. 4) the isotropic liquid crystalline phase was found at high emulsifier concentrations and the emulsion stability is gradually increased reaching high values at emulsifier concentrations of 15 % and above.

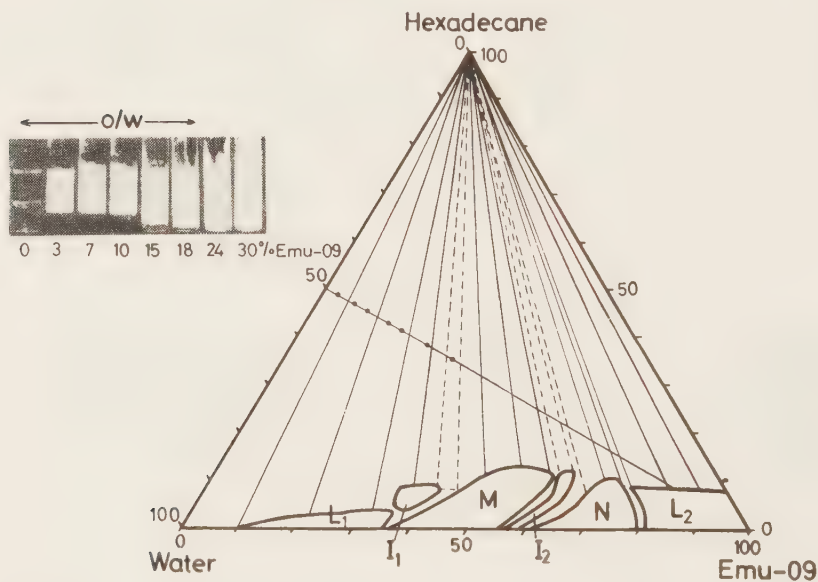


Fig. 4
Phase equilibria and emulsion stability for the system Emu-09/
Hexadecane/Water

Discussion

The results showed the importance of phase equilibria for emulsion stability. Emulsion stability was high when the system contained a liquid crystalline phase in addition to the aqueous and oil phases. When the emulsifier concentration was increased and the system passed from the three-phase area to a two-phase area containing the oil and liquid crystalline phases, the stability was reduced. Such low stability emulsions with high percentages of emulsifiers were found in all systems except the one in Fig. 4. In this case, the

isotropic liquid crystalline phase, which is responsible for the enhanced emulsion stability, showed a very high viscosity. In our opinion this property has a pronounced influence on the emulsion stability.

The results also demonstrated the influence of emulsifier impurities on the phase behaviour in these systems and consequently also on emulsion properties. In general, the unpurified emulsifier gave rise to more stable emulsions at low emulsifier concentrations. A pronounced change in phase behaviour took place when the p-xylene was replaced by hexadecane (Figs. 3 and 4). The change was also accompanied by corresponding alterations in emulsion stability. In our opinion these results offer an explanation to the problem of the influence of different hydrocarbons stated earlier by Davies /10/. His opinion that emulsions were stabilized by a gelly "skin" agrees well with our results showing a third phase of liquid crystalline nature to be present.

The questions concerning liquid crystalline phases in emulsions which remain to be answered are concerned with the specific mechanism of the protection of the liquid crystalline phase and with the nature of the transient liquid crystalline structures present only in the emulsified, nonequilibrium state of the system. From calculations of the van der Waals interaction between the droplets using Vold's approach with refined expressions for the geometric interaction factor it appears probable that the reduction is insignificant. The viscous forces which have to be overcome in order to obtain coalescence appear, however, to be of a more important magnitude.

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Discussion

Question by K. Shinoda

I appreciate your precise discussions based on the phase equilibria. You are dealing with 3 component system. If you have 3 phases, two are oil phases and a water phase and the last one must be surfactant phase. Whether the surfactant phase is either isotropic or anisotropic is depending on the temperature of the system.

Answer

At room temperature the third phase is a liquid-crystalline phase. At high temperatures it appears probable that the liquid crystalline phase will form a liquid isotropic phase. The liquid crystalline phase at room temperature may be optically isotropic. Its liquid crystalline character is demonstrated by the X-ray diffractograms showing long-range order and short-range disorder.

Question by B. Tamamushi

What do you think about the difference between aliphatic and aromatic hydrocarbons concerning the emulsion stability. Is it not due to the steric factors of molecular forms ?

Answer

It appears that the steric factors have a high degree of importance. A phenol emulsifier shows completely different packing conditions with an aromatic hydrocarbon in the liquid crystalline phase than with an aliphatic hydrocarbon.

Question by Th.F.Tadros

Have you investigated the effect of temperature on emulsion stability ? Certainly if stability is due to a high viscous force of the liquid crystalline phase, temperature will have a significant effect on stability.

Answer

Temperature certainly effects the stability. We have not investigated these phenomena which were determined in extensive research by Prof. Shinoda from the beginning of the sixties.

Question by A.A. Trapeznikov

1. What is the structure of a stable emulsion, when the emulsifier is in a liquid-crystalline state and what is the thickness of a stabilizing layer ?
2. What is the viscosity of the emulsifier when it is in a liquid-crystalline state on a surface of drops ?

Answer

1. Our knowledge so far indicates that the liquid droplets are surrounded by a layer of the liquid crystalline phase. In our experience the amount of liquid crystalline phase present has to be at a level corresponding to at least a thickness of 500 Å.
2. The viscosity varies, but generally exceeds 500 CP.

Question by K. Shinoda

If you change the composition of the system, you may obtain two phase systems, i.e. oil and surfactant, oil and water, or water and surfactant. So, the emulsion is not always O/W or W/O, but O/S, S/O, W/S or S/W type as well.

Answer

Emulsions containing only one liquid phase dispersed in a liquid crystalline phase or vice versa may be obtained.

Question by A.G. Kanellopoulos

Does the emulsification method have any effect on the emulsion stability ?

Answer

The emulsification method influences the stability of the emulsion to a high degree; especially at low concentrations of the emulsifier.

PAPER III

The Importance of the Phase Behaviour of Phospholipids for Emulsion Stability

By *L. Rydhag**, The Swedish Institute for Surface Chemistry, Stockholm

The relationship between the phase behaviour for different combinations of neutral and charged surface active lipids was investigated with regard to the dispersion and stabilization of emulsified systems. When the negatively charged lipids were combined with neutral phospholipids lamellar liquid crystalline phases were formed with large repeat-distances depending on the incorporation of water between the lipid bilayers. Studies of phase equilibria in a water-oil-phospholipid system showed that the lamellar phase was present in that concentration area where the o/w emulsions investigated were produced. The investigation made clear that both the degree of dispersion and the emulsion stability could be brought to optimum values by the addition to neutral phospholipids of negatively charged lipids or by selecting commercial lecithins in which a certain amount of negatively charged phospholipids were present.

Introduction

Emulsifiers are added to emulsions partly in order to facilitate the breaking up of the disperse phase partly in order to increase the stability of the emulsion or because certain emulsifiers preferentially cause the formation of o/w or w/o emulsions. Phospholipids are permitted as food emulsifiers, but they also occur as emulsifiers in pharmaceutical and cosmetical preparations. In the lecithins of commercial origin used in foodstuffs both the composition and the total concentration of the phospholipids vary according to the supplier but variations between batches also occur.

* Author's address: *Lisbeth Rydhag*, The Swedish Institute for Surface Chemistry, Box 56 07, S-114 28 Stockholm.

Die Bedeutung des Phasenverhaltens von Phospholipiden für die Emulsionsstabilität

Die Beziehung zwischen dem Phasenverhalten unterschiedlicher Kombinationen von neutralen und geladenen oberflächenaktiven Lipiden wurde in Hinblick auf Dispergierung und Stabilisierung von emulgierten Systemen untersucht. Bei der Kombination der negativ geladenen Lipide mit neutralen Phospholipiden entstanden lamellare flüssig-kristalline Phasen mit großen Wiederholabständen in Abhängigkeit von der Inkorporation des Wassers zwischen den Lipid-Doppelschichten. Untersuchungen der Phasen-Gleichgewichte in einem Wasser-Öl-Phospholipid-System zeigten, daß die lamellare Phase in derjenigen Konzentration vorliegt, bei der die Öl-Wasser-Emulsionen gebildet werden. Die Untersuchungen zeigten deutlich, daß sowohl das Ausmaß der Dispergierung als auch die Emulsionsstabilität durch Zusatz von negativ geladenen Lipiden zu den neutralen Phospholipiden oder durch geeignete Auswahl von handelsüblichen Lecithinen mit einem gewissen Anteil an negativ geladenen Phospholipiden auf optimale Werte gebracht werden können.

Soybean lecithin is obtained as a by-product in the production of oil from soybeans. The phospholipids are removed from the raw soybean oil in a degumming procedure, i.e. those components which swell with water produce a liquid crystalline phase which is removed from the oil. After evaporation of the water the mixture contains about 65% phospholipids and about 35% raw soybean oil and is then known as the commercially available soybean lecithin.

Depending on both the history of the soybeans themselves and the method used for their extraction, the soybean lecithin will vary both as to the composition and to the total concentration of the phospholipids. In certain cases the soybean lecithins designed for use in

pharmaceutical or cosmetic preparations or in health foods are further refined by fractionation or de-fatting.

The purpose of the investigation was to attempt to elucidate the relationship between the composition of the surface-active phospholipids and their phase behaviour in water-oil systems and also their functions in connection with the dispersion and stabilization of oil-in-water emulsions.

In the preliminary stage of the investigation the commercial lecithins used were characterized with respect to the composition and total concentration of the phospholipids, whereby the swelling properties of certain phospholipid mixtures were investigated by means of their low angle X-ray-diffraction patterns. The phase equilibria were then determined for a water-oil-phospholipid system, after which emulsions were prepared under controlled conditions in a multicomponent system with known phase behaviour. The swelling properties of the phospholipid mixtures were matched in a model system in which the importance of the negatively charged lipids for the degree of dispersion and for emulsion stability could be investigated.

Materials and Methods

Both phosphatidyl choline (PC), well defined with respect to the polar group, and mixtures of phospholipids with a fatty acid pattern corresponding to that of soybean oil were used. In the mixed phospholipid preparation, which was a defatted soybean lecithin of commercial origin, the amounts of constituent phospholipids were determined according to a previously described method¹. After two dimensional TLC-separations on silica gel plates, first with a basic eluent and then an acid one, regions on the plate corresponding to phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), phosphatidyl inositol (PI), phosphatidyl serine (PS), and phosphatidic acid (PA) were analysed quantitatively for phosphorous according to the method described by R. R. Lowry and F. J. Tinsley². Of the commercial lecithins, only those phospholipids were used which were precipitated by treatment with acetone. The fatty acid pattern of the lecithins employed can be seen in Table 1.

Table 1

A characteristic fatty acid pattern of a soybean phosphatidyl choline, determined by means of the gas-chromatographic analysis of the methyl esters according to R. L. Glass et al.³

C ₈	0.8 %	C ₁₈₌₁	10.7 %
C ₁₆	12.2 %	C ₁₈₌₂	67.2 %
C ₁₆₌₁	0.4 %	C ₁₈₌₃	6.0 %
C ₁₈	2.7 %		

Since the soybean lecithins contained very unsaturated fatty acids all the investigations were made above the chain melting temperature which was determined to $\sim -20^{\circ}\text{C}$.

The salts used were of p.a. quality, the sodium stearate was synthesized at the Institute, all water was

twice distilled and the oil used was refined, deodorized soybean oil, SBO.

The abbreviations PC, PE, PI, PA and PS are used for phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl inositol, phosphatidyl acid and phosphatidyl serine, respectively. PC and PE were included among the neutral phospholipids at pH 7, while PI, PA and PS were considered as negatively charged.

Phase equilibria

The phospholipids were dissolved together with the oil in petroleum ether and ethanol, after which the solvent was evaporated under reduced pressure. Water was then added, after which the mixture was repeatedly pressed through a centrifuge tube with a narrow constriction or a similar arrangement of two syringes. The phases were then separated by ultracentrifugation 150 000 xg and identified by means of their low angle X-ray diffraction patterns at 20°C . The samples were handled in a nitrogen atmosphere on account of the high degree of unsaturation in the fatty acid chains of both the phospholipids and the oil. Otherwise the determinations were carried out according to the previously described methods applicable to ternary system^{4,5}.

Emulsification and the assessment of emulsion stability

Emulsions of varying oil:water ratios and with emulsifier concentrations from 0.5 to 3% were prepared in a valve homogenizer⁶ under controlled conditions, i. e. length of time, pressure and temperature were kept constant. Before emulsification the phospholipids were dispersed in oil, after which the oil phase was mixed with the water phase in the homogenizer. Afterwards the emulsion was transferred to a graduated glass cylinder and kept at constant temperature (20°C). Emulsion stability was judged partly by visual examination when the separation of the oil and water phases was recorded, partly by the determination of droplet size in the upper and lower part of the sample volume respectively first immediately after the preparation of the emulsion and then again after a certain interval of time. The droplet size determination was carried out by means of a Coulter Counter, Model TA, Coulter Electronics Ltd., England. Flocculation was examined with the help of a light microscope equipped with polarization filters and interference contrast. The electrophoretic mobility of the droplets was determined by means of a Micro-Electrophoresis Apparatus Mark II, Rank Bros., Bottisham, Cambridge, England. Emulsion type was determined under the microscope after staining the water and oil phases with methylene blue and Sudan red, respectively. The emulsions were judged to be stable when neither phase separation, flocculation or coalescence had taken place after a period of five days.

Results

Phase equilibrium

For an arbitrarily chosen soybean phospholipid mixture, SBP, with the composition shown in Table 2,

⁴ K. Fontell, L. Mandell, H. Lehtinen and P. Ekwall, *Acta Polytech. Scand. Chem.* **74**, 1 [1968].

⁵ L. Mandell and P. Ekwall, *Acta Polytech. Scand. Chem.* **74**, 1 [1968].

⁶ H. Mulder and P. Walstra, "The Milk Fat Globule", 1974, Pudoe, Wageningen, and Commonwealth Agric. Bureaux, Farnham Royal.

¹ A. Biverstedt and L. Rydhag, 9th Scand. Lipid Symposium, Visby 1977.

² R. R. Lowry and F. J. Tinsley, *Lipids* **9**, 7 [1974].

³ R. L. Glass, R. Jenness and H. A. Troolin, *J. Dairy Sci.* no 8 [1965].

swelling with water was determined by low angle X-ray diffraction investigations. The results showed that a lamellar phase was formed when the phospholipid mixture, SBP, was swelled with water. The swelling corresponds to the ideal case of a lamellar phase incorporating all the water of the system. About 70 per cent of water was incorporated in the lamellar phase. In its natural state the phospholipid mixture contained 35 mol per cent anionic lipids in the form of PI and PA. For comparison purposes the swelling of a well defined phosphatidyl choline, PC, with water was determined under the same conditions. It can be seen from Figure 1 that PC swelled to a lamellar phase incorporating rather less than 50%, after which the same repeat distance, approx. 64 Å, was obtained for the lamellar phase despite the increased concentration of water in the samples.

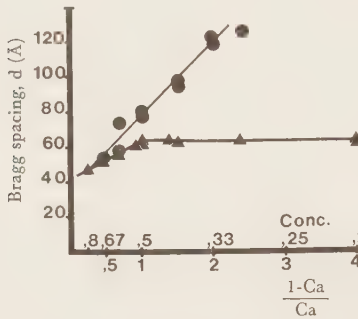


Fig. 1. X-ray diffraction spacings from phospholipid — water mixtures as a function of the lipid concentration —●—●— SBP — water; —▲—▲— PC — water

In order to obtain a swelling in water, corresponding to that of the natural phospholipid mixture SBP, an anionic lipid was added, sodium stearate. The soap was dissolved in the phosphatidyl choline (PC) by means of petroleum ether and alcohol. The samples were then prepared as previously mentioned. *T. Gulik-Krzywicki et al.*⁷ reported 1969 that such additives distinctly increased the incorporation of water for the lamellar phase

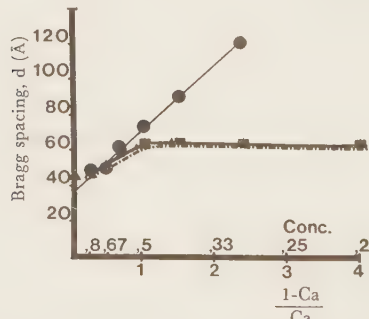


Fig. 2. X-ray diffraction spacings from phospholipid — water mixtures as a function of the lipid concentration —▲—▲— PC — water —●—●— PC/sodium stearate — water —■—■— PC/stearic acid — water

⁷ *T. Gulik-Krzywicki, A. Tardieu and V. Luzzati, Molec. Cryst. and Liq. Cryst. 8, 285 [1969].*

formed by egg lecithin. For the soybean phosphatidyl choline (PC) here involved the addition of the soap caused an increased swelling with water which was not obtained by the addition of stearic acid (Fig. 2). The swelling corresponds almost to the ideal case of swelling of a lamellar phase. Since foodstuffs are generally considered to contain approx. 1% sodium chloride, the effect of such an addition upon the swelling of the phospholipid (PC) was also investigated. From Figure 3 it

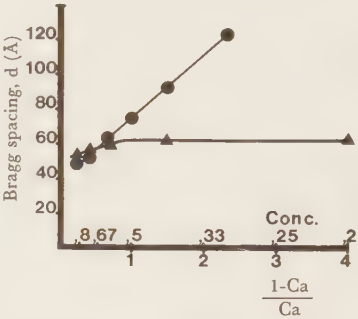


Fig. 3. X-ray diffraction spacings from phospholipid — water mixtures as a function of the lipid concentration —●—●— PC/sodium stearate — water —▲—▲— PC/sodium stearate — 1% sodium chloride solution

is evident that the lipid layer, swollen by the addition of the soap, was compressed when the water was replaced by a 1% sodium chloride solution.

The solubility of soybean oil, SBO, in the lamellar liquid crystalline phase was low for the phospholipid mixture, SBP. The one, two and three-phase areas are evident from the phase diagram in Figure 4. On the

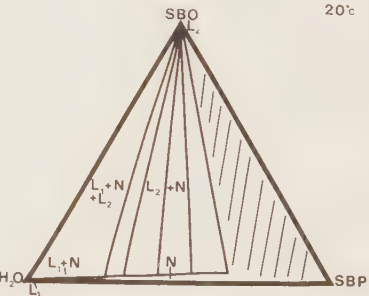


Fig. 4. Ternary phase diagram of water — phospholipid mixture (SBP) — soybean oil (SBO) at 20°C
 L₁ is the water solution of SBP in water
 L₂ is the solution of SBP in soybean oil
 N is the lamellar liquid crystalline phase

addition of SBP to oil and water the emulsifier is precipitated in the form of a liquid crystalline phase (N) existing in equilibrium with an aqueous solution of SBP (L₁) and a solution of SBP in oil. The solubility of egg lecithin in water is stated by *C. Tanford*⁸ to be approx. 5×10^{-10} M. For SBP the solubility in soybean oil was determined to be 0.03%, i.e. about 4×10^{-3} M. Since

⁸ *C. Tanford, in: The Hydrophobic Effect, John Wiley & Sons, New York 1973.*

the solubilities of SBP and PC in both water and oil, respectively are low, this means that nearly all of the emulsifier precipitated in the form of a lamellar phase (N). The three component system water-soybean oil-PC is shown in Figure 5. The solubility of soybean oil, SBO,

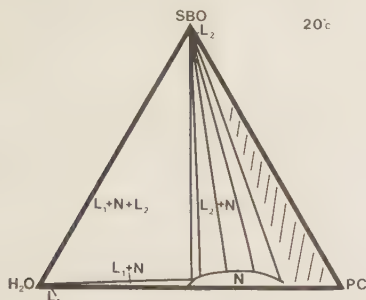


Fig. 5. Ternary phase diagram of water — phosphatidyl choline (PC) — soybean oil (SBO) at 20°C
 L_1 is the water solution of PC
 L_2 is the solution of PC in SBO
 N is the lamellar liquid crystalline phase

increased somewhat in this system but still lies below 10%. The phosphatidyl choline also formed, in this system, a lamellar phase (N) in equilibrium with an aqueous solution (L_1) and an oil solution (L_2) of PC.

Only those parts of the phase diagram which contain high water concentrations were investigated thoroughly because the emulsions contained relatively small amounts of emulsifiers.

Emulsions

Emulsions with varying oil:water ratios were prepared with emulsifier contents from 0.5 to 3 weight-%.

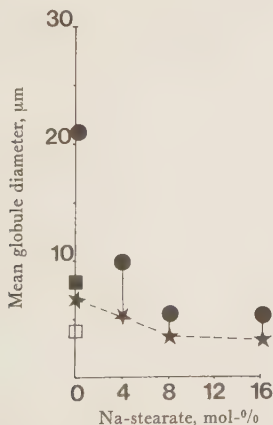


Fig. 6. The mean globule diameter as a function of the amount of negatively charged lipid added to the emulsifier
 ★ mean globule diameter, directly after preparation, emulsifier PC
 ● mean globule diameter after five days' storage, emulsifier PC
 □ mean globular diameter, directly after preparation, emulsifier SBP
 ■ mean globular diameter after five days' storage, emulsifier SBP

In the systems investigated only o/w emulsions were formed. The dispersing and stabilizing properties of the different phospholipid preparations were compared in an arbitrarily chosen series of emulsions containing 40:60 oil to water phase ratio. The concentration chosen for the emulsifier was 1% since quite stable emulsions could be prepared at this concentration. The results are summarized in Figure 6, which shows the mean values for the droplet size, determined both immediately after preparation and also after 5 days, plotted against the amount of negatively charged lipid added to the emulsifier. From Figure 6 it is evident that addition of negatively charged lipid on the one hand reduced the initial droplet size while also reducing, at the same time, the tendency of the emulsion to coalesce. The total interfacial area formed in the emulsion between oil and water and between oil and salt solution are shown in Figure 7. Addition of sodium chloride to

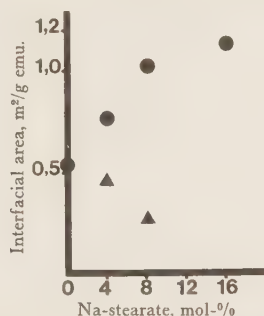


Fig. 7. The interfacial area created per gram of emulsion as a function of the amount of negatively charged lipid added to the emulsifier PC
 ● — interfacial area of emulsions in the PC — sodium stearate — water system
 ▲ — interfacial area of the emulsion in the PC — sodium stearate — 1% sodium chloride solution

the water phase resulted in a marked reduction of the total interfacial area formed in the emulsion, while at the same time the stability of the emulsion was affected negatively. (Compare the effect of the salt on the repeat distances in Figure 3). The thickness of the phospholipid film was calculated on the assumption that all surface-

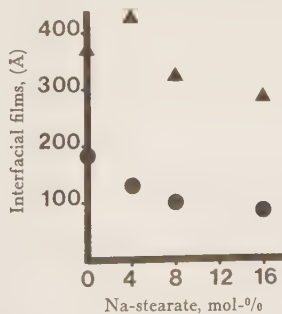


Fig. 8. The estimated thickness of the interfacial films of phosphatidyl choline as a function of the amount of charged lipid in the emulsifier, PC
 ● — nonhydrated phospholipid film
 ▲ — hydrated phospholipid film

active lipid was located at the oil-water total interfacial area of the emulsion, Figure 8. The thickness of the film varied between approx. 100 Å and 200 Å depending on the amount of negatively charged lipids in the emulsifier. The dimensions of the hydrated phospholipid films were calculated from the knowledge of the phospholipids swelling properties (see Fig. 2). For bimolecular phos-

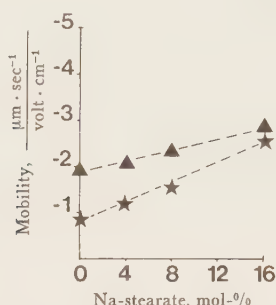


Fig. 9. The electrophoretic mobility was determined in o/w-emulsions containing 1% of emulsifier and with an oil: water ratio of 40:60

—▲— the electrophoretic mobility as a function of the amount of charged lipid in the emulsifier, PC. The water phase was a 0.1% sodium chloride solution
 —★— the electrophoretic mobility as a function of the amount of charged lipid in the emulsifier. The water phase was a 0.02 M Tris-HCl solution, pH 7.4

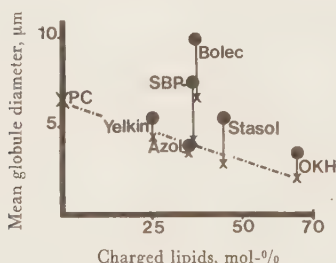


Fig. 10. The mean globule diameter as a function of the amount of charged phospholipids in the emulsifier
 × mean globule diameter, directly after the preparation
 ● mean globule diameter after 5 days' storage

phatidyl choline layers the Bragg spacings can be calculated, from Figure 2, to be $d \approx 40$ Å, which is in agreement with the values given in the literature⁹. The mobility of the emulsion droplets increased with increased addition of negatively charged lipid in the emulsifier (Fig. 10). The determinations were made, partly in 0.1% NaCl solution, partly in 0.02 M Tris-HCl buffer at pH 7.4. Corresponding investigations of emulsions where the emulsifier consisted of phospholipids extracted from commercial soybean lecithin mixtures, showed a comparable relationship between droplet size and the naturally occurring amount of negatively charged phospholipids. The droplet size was determined immediately after preparation, and also after 5 days' storage, for 40:60, oil:water emulsions stabilized with 1% mixed phospholipids, Figure 10. The tendency to coalesce varied considerably irrespective of the content of anionic lipids, while a better correlation was obtained of the initial droplet size in the emulsions with the concentration of anionic lipids. The phospholipid contents of the commercial lecithins are recorded in Table 2.

The most effective dispersion was obtained with those mixed phospholipids which had the largest contents of negatively charged phospholipids. Little coalescence was recorded for emulsions stabilized with Yelkin, Azol and OKH. Compared with the phosphatidyl choline (PC) all of the commercial lecithins investigated were superior both with respect to dispersion and to stabilisation of the o/w emulsions investigated.

The mobility of the drops was determined by particle electrophoresis and the relationship between the mobility and the negatively charged phospholipids in the lecithin is evident from Figure 11, which shows that a good correlation was obtained for the mobility of the disperse phase in relation to the amount of anionic lipids in the emulsifier. The mobility of the disperse phase became greater with increased concentrations of anionic lipids.

Discussion

Those phospholipids which are used as foodstuff emulsifiers are insufficiently defined with respect both

* D. M. Small, J. Lipid Res. 8, 551 [1967].

Table 2

The Phospholipid Composition of the Commercially Available Soybean Lecithins

Producer	Trade name	PC mol %	PE mol %	PI mol %	PS mol %	PA mol %	Lyso mol %	Anionic lipids mol %	P _{tot} * wt %
Ross & Rover Inc. N Y, USA	YELKIN	41	34	19	—	6	—	25	49
Staley Manuf. Co., USA	STASOL 3318	34	10	29	—	12	15	41	65
Unimills, Hamburg, Germany	Bolec Z	33	29	24	—	14	—	38	51
Lucas Meyer, Hamburg, Germany	AZOL	45	19	11	25	—	—	36	41
Aarhus Oliefabr., Denmark	OKH	11	19	41	—	29	—	70	68
Unknown, Netherland	SBP-BZ	33	32	21	—	14	—	35	78
Lucas Meyer, Hamburg, Germany	Epikuron-200, PC	100	—	—	—	—	—	—	98

* total phospholipids in products
 calc. mol. wt = 750

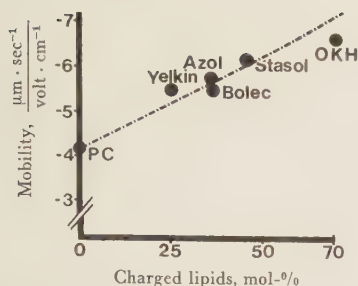


Fig. 11. The electrophoretic mobility of the oil droplets as a function of the amount of negatively charged phospholipids in the emulsifier

to phospholipid concentrations and phospholipid content and to their function as emulsifiers. Very extensive investigations have been carried out, on the other hand, on membrane lipids, and this applies both to the isolated lipids and to mixtures of these⁹⁻¹³. The difference between phospholipids from soybean and those from egg or from biological tissue consists only in that the soybean phospholipids have more unsaturated fatty acids than the corresponding phospholipids from egg or biological tissue. With unsaturated fatty acids it follows that the chain melting temperature of the phospholipid is low compared with that of phospholipids with saturated fatty acid chains¹⁰. For the phospholipids used in the investigation the chain melting point is far below room temperature.

Several investigations show that egg lecithin only gives one lamellar phase^{9, 12, 13}. With low water contents, however, several phases are formed with different packing from the lamellar¹¹. Most naturally occurring membranes contain 20–30% anionic lipids, compared with 70–80% neutral or zwitterionic lipids⁸. Several of the membrane lipid mixtures swell with water to give lamellar phases with large repeat distances¹³. The soybean phospholipid mixtures investigated contained between 25 and 41 mol per cent anionic lipids (Tab. 2). Since the swelling behaviour for the natural membrane lipids can be imitated in a model system with well defined components the dispersion and emulsion stabilities were examined for the four-component system water-oil-neutral lipids-negatively charged lipids. *T. Gulik-Krzywicki* et al. found that additions of positively or negatively charged soaps to egg lecithin resulted in an ideal swelling of the lamellar phase incorporating large amounts of water⁷.

The addition of electrolytes to the swelled lamellar phase, incorporating large amounts of water, reduced the swelling to that of the PC-water system (Fig. 3). Similar results concerning the addition of electrolytes

were found by *T. Gulik-Krzywicki* et al.⁷. The addition of electrolytes to the water phase, 1% of NaCl, influenced the dispersion and the emulsion stability negatively. The reduction of the total interfacial area is shown in Figure 7.

It is well-known that lamellar phases in water-soap systems can be stabilized by the presence of amphiphilic substances such as fatty alcohols or fatty acids¹⁴. *M.-R. Hakala*, *J. B. Rosenholm* and *P. Stenius*¹⁵ have in a recently published paper pointed out that the interaction between the hydroxyl and the carboxylic groups is especially important in stabilizing the lamellar phase containing large amounts of water.

In experiments in the previously mentioned four-component system we found that optimal dispersion and stabilization was obtained when emulsifiers swelled with water to give large repeat distances. Increased amounts of charged lipids are also reflected in the surface charge of the disperse phase. Those experiments carried out with PC alone are reported to have given unstable emulsions¹⁶. On the assumption that the surface active lipids are adsorbed at the oil-water interface, the film thickness for this lipid layer was calculated (Fig. 8). Although the area of the total interfacial area is always underestimated because of the difficulty of counting the number of drops less than one microne in size, the available phospholipids seem to form at least one lipid bilayer at the oil-water interface. The occurrence of multilamellar liquid crystalline phases in emulsions and their importance with respect to emulsion stability have earlier been pointed out by *S. Friberg* and coworkers^{17, 18}.

According to the phase diagrams, for all the emulsions investigated there were lamellar liquid crystalline phases in the concentration regions where the emulsions were produced. Unstable emulsions were formed, however, when PC alone was used as emulsifier, while both dispersion and stabilization increased with increased amounts of negatively charged lipids in the emulsifiers.

Acknowledgments

Inga Wilton with whom I have had many interesting and stimulating discussions.

Ann-Marie Wennerberg and *Inger Larsson* who prepared the emulsions and determined the emulsion stability.

Arne Biverstedt and *Thomas Gabrán* who characterized the phospholipids.

The project was financed by a grant from the Swedish Board for Technical Development.

Received 24th April 1978.

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PAPER IV

✿ The Function of Phospholipids of Soybean Lecithin in Emulsions¹

L. RYDHAG and I. WILTON, The Swedish Institute for Surface Chemistry, Box 5607, S-114 86 Stockholm, Sweden

ABSTRACT

A number of commercially available soybean lecithins were analyzed with respect to their phospholipid composition and emulsifying properties. A phosphatidylcholine (PC) from soybean swells to a lamellar liquid crystalline phase which incorporates slightly less than 50% of water. The swelling behavior of the commercially available soybean lecithins may be different depending on the concentration of other phospholipids such as phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidic acid (PA). In the presence of the negatively charged phospholipids PI and PA, the swelling of the lamellar phase of PC was dramatically enhanced while a lecithin with equal amounts of PC and PE and small quantities of PI and PA formed two liquid crystalline phases, i.e., a lamellar and a hexagonal phase. Stable o/w-emulsions can be prepared when the phospholipid composition is such that a lamellar liquid crystalline phase in equilibrium with the oil and water phases incorporates large amounts of water. The minimal amount of emulsifier required to stabilize the emulsions has been estimated to give an interfacial film of ca. 80 Å thickness which corresponds to a thickness of two double lipid layers in the interfacial film. The incorporation of large amounts of water is obtained if the lamellar layers contain dissociated ionic groups.

INTRODUCTION

Phospholipids from soybeans are permitted as food emulsifiers and they are also used as emulsifiers in pharmaceutical, cosmetic and technical preparations. Different compositions and total concentrations of phospholipids are declared by different suppliers of lecithins of commercial origin used in food. However, we have shown that large variations between batches occur (1).

Soybean lecithin is obtained as a by-product in the production of oil from soybeans. The phospholipids are removed from the raw soybean oil in a degumming procedure, i.e., those components which swell with water precipitate as a liquid crystalline phase which is removed from the oil. After evaporation of the water, the precipitated mixture contains ca. 65% phospholipids and ca. 35% raw soybean oil. This is what is known as the commercially available soybean lecithin. Depending on both the history of the soybeans themselves and the method used for their extraction, the soybean lecithin will vary both as to the composition and the total concentration of the phospholipids whereas the fatty acid pattern corresponds to that of the soybean oil.

It would obviously be highly desirable to establish whether the variability in composition of commercially available lecithins can be correlated with their properties as emulsifiers. In this paper, we report continued studies of the swelling behavior of different lecithins and show that this may be correlated to their composition as well as to their function as emulsifiers.

We have studied three commercial lecithins. Their phospholipid composition and fatty acid pattern were determined and are reported in Materials and Methods. The swelling properties were investigated by means of low angle X-ray diffraction patterns. Phase equilibria were determined

for a water-soybean emulsifier system. Knowledge of these made it possible to prepare emulsions under controlled conditions in a multicomponent system for which the phase equilibria were known. Emulsion stabilities were determined as a function of the o/w ratio and the amount of emulsifier in the model systems. We will show that, in addition to the correlation between phase equilibria and emulsion properties, the studies give some important insights into the stabilization mechanism of the o/w emulsions.

MATERIAL AND METHODS

Methods of Analysis

The emulsifiers were all of commercial origin, i.e., phosphatidylcholine (PC), well defined with respect to the polar group, and mixtures of phospholipids with a fatty acid pattern corresponding to that of soybean oil. In the mixed phospholipid preparations, the amounts of constituent phospholipids were determined according to the method described (1). After two-dimensional thin layer chromatographic (TLC) separations on silica gel plates, first with a basic eluent and then an acid one, spots on the plate corresponding to PC, phosphatidic acid (PA) and lysophosphatides were identified but only PC, PE, PI and PA were analyzed quantitatively for phosphorous according to the method described by Lowry and Tinsley (2). The amount of lysophosphatides was less than 2 mol %. The phospholipid compositions are shown in Figure 1.

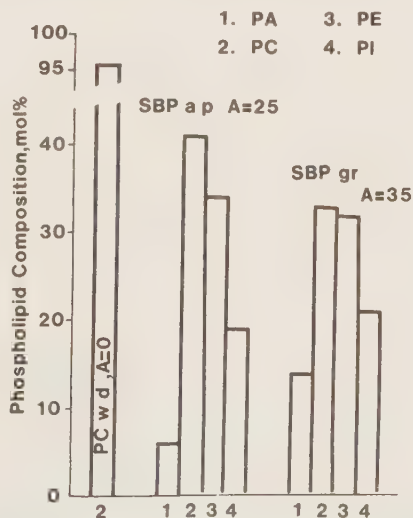


FIG. 1. The principal phospholipid composition of the soybean lecithins which were used as emulsifiers.

¹ Presented at the ISF/AOCS World Congress, New York, April 1980.

TABLE I^a

Product	Fatty acid composition (wt. %)				
	C ₁₆	C ₁₈	C _{18:1}	C _{18:2}	C _{18:3}
Phosphatidylcholine, well-defined (PCwd)	14.9	3.2	13.7	62.7	5.2
Soybean phosphatides, acetone-precipitated (SBPap)	21.5	4.3	7.2	60.9	6.1
Soybean phosphatides, granulated (SBPgr)	18.9	4.1	6.8	60.8	9.2
Soybean oil, refined, deod. (SBO)	11	4	25	50	8

^aThe fatty acid pattern of the soybean phosphatides and the soybean oil varies. The table shows the composition for the batches used in this investigation.

The fatty acid patterns of the soybean PC, the mixed phospholipid preparations and the soybean oil were determined by means of gas chromatographic analysis of the methyl esters according to Glass et al. (3). The results are summarized in Table I.

The influence of the batch variation of the phospholipid composition of a commercial lecithin has earlier been reported (1). The amount of negatively charged phospholipids varied, e.g., between 29 and 42 mol % in 6 different batches of an acetone-precipitated commercial lecithin.

Definition of A-Value

The pK_a values of PA are $pK_1 = 3.8$ and $pK_2 = 8.6$ in water at 25°C (4). Hence, while the PC and PE are neutral at pH 6-7 the PA and PI are partly dissociated and, thus, negatively charged, each molecule carrying one charge. The fraction of negatively charged phospholipids thus may be expressed as the sum of mol % (A) of PA and PI.

$$A = \frac{n_{PA} + n_{PI}}{n_{PA} + n_{PC} + n_{PE} + n_{PI}} \times 100,$$

where n_i is the amount of component i .

Emulsifiers

(a) PC wd is a well defined soybean lecithin containing 98 mol % PC (i.e., $A \approx 0$) delivered by Lucas Meyer, Hamburg, Germany. (b) SBPap is an acetone precipitation of a Yelkin® lecithin from Ross & Rowe, NY, and contains 90% phospholipids with $A = 25$. (c) SBPgr is a defatted and granulated lecithin of unknown origin in The Netherlands. It contains 78% phospholipids with $A = 35$.

Soybean lecithins contain mostly unsaturated fatty acids and hence all investigations have been made well above the chain melting temperature (cmt) which was -17°C for the well defined soybean PC in water.

The salts used were of p.a. quality, all water was twice distilled and the oil used was refined, deodorized soybean oil (SBO).

Phase Equilibria

The phase equilibria in the pseudo-ternary system water/oil/phospholipid were determined thus: The phospholipids were dissolved together with the oil in petroleum ether and ethanol, after which the solvent was evaporated under reduced pressure. Following the addition of water, the mixture was repeatedly forced through a centrifuge tube with a narrow constriction or a similar arrangement of two syringes. The phases were then separated by ultracentrifugation at 150,000 G and identified by means of their low angle X-ray diffraction patterns at 20°C. The samples were handled in a nitrogen atmosphere because of the high degree of unsaturation in the fatty acid chains of both the

phospholipids and the oil. Otherwise, the determinations were carried out according to previously described methods that have been used for ternary systems (5,6).

Emulsification Procedure

Emulsions of varying oil:water ratios and with emulsifier concentrations from 0.1 to 3% were prepared in a continuously working valve homogenizer under controlled conditions, i.e., time, pressure and temperature were kept constant. The apparatus is a modified hand-powered valve homogenizer which was improved by Tomberg and Lundh at the Technical University of Lund, Sweden (7). Before emulsification, the phospholipids were dispersed in the oil phase, after which the oil was mixed with the water phase in the homogenizer. Afterwards, the emulsion was transferred to a graduated glass cylinder and kept at constant temperature (20°C).

Characterization of Emulsions

The following terms describing different steps in the aggregation processes involving emulsion droplets will be used. *Creaming*: the dispersed oil phase floatate; *flocculation*: two or more droplets form an aggregate but keep their identity; *coalescence*: two or more droplets form a bigger one.

Emulsion stability was judged (a) by visual determination of the separation of the oil and water phases, and (b) by the determination of droplet size in the upper and lower parts of the sample volume immediately after the preparation of the emulsion as well as after 24 hr. Coalescence and creaming were determined by means of visual observation combined with drop size determinations. The droplet size determination was carried out by means of a Coulter Counter, Model TA, Coulter Electronics, Ltd., England. Flocculation was examined with the help of a light microscope equipped with polarization filters and interference contrast. Emulsion type was determined under the microscope after staining the water and oil phases with methylene blue and Sudan red, respectively. The emulsions were judged to be stable when neither phase separation, creaming, flocculation or coalescence had taken place after a period of 24 hr.

RESULTS

Phase Equilibria

X-ray diffraction measurements. The swelling behavior of the acetone-precipitated phospholipid mixture, SBPap in water, was investigated by means of low angle X-ray diffraction at 25 and 40°C. The higher temperature was chosen to check that all the saturated fatty acid chains of the SBPap were in a fluid state. The swelling was identical at 25 and 40°C.

A liquid crystalline phase with two-dimensional hexagonal symmetry characterized by Bragg spacings in the ratio of $1:1/\sqrt{3}:1/\sqrt{4}$ was observed at low water concentrations. This phase is a reversed hexagonal structure (RM), type F according to Fontell et al. (6) or H_H according to Luzzati (8). At water concentrations larger than 20%, a phase was formed which was characterized by Bragg spacings in the ratio of $1:1/2:1/3$. This corresponds to a lamellar phase (N), D according to Fontell et al. or L_α according to Luzzati. Figure 2 shows the repeat distance d of the liquid crystalline phases as a function of $1-C_a/C_a$, where C_a is the phospholipid concentration which is proportional to the inverse volume fraction of the phospholipids. As expected for a lamellar liquid crystalline phase, the d value increases linearly with this quantity between 20 to 64 wt. % water. The swelling corresponds to the case of

a lamellar phase incorporating all the water of the system. When the water concentration exceeds 64 wt. %, two phases exist in equilibrium, i.e., the lamellar phase (N) and a very dilute aqueous solution of the phospholipid mixture.

The swelling behavior and the phase equilibria have been established earlier for the well defined soybean PC, PCwd, and the granulated phospholipid mixture (9). The swelling of the three phospholipids are compared in Figure 3.

The PCwd swells to a lamellar phase which incorporates slightly less than 50 wt. % water. At larger water concentrations, two phases exist in equilibrium, the lamellar phase and a solution of the PCwd monomers in water. At maximal swelling with water the repeat distance is $d = 64 \text{ \AA}$ (Fig. 3). The lamellar phase formed by the SBPap containing 25 mol % of charged lipids incorporates a maximum of 65 wt. % water. The repeat distance at this maximum

was $d = 124 \text{ \AA}$. The SBPgr with 35 mol % of charged lipids also forms a lamellar phase containing 70 wt. % water at maximal swelling. The repeat distance, in this case, was also $d = 124 \text{ \AA}$.

Thus, the presence of charged phospholipids in the phospholipid mixtures renders the lamellar phases capable of incorporating larger amounts of water than the well defined phospholipid which contains undissociating molecules only.

The thickness of the lipid bilayer (d_1) may be obtained by extrapolation of the expression $1 - C_a/C_a$ to zero concentration of water. d_1 is ca. $40 \text{ \AA}$ for all the emulsifiers used in this investigation. The slopes of the repeat distance to inverse volume concentration plots is slightly different for the mixed phospholipid preparations.

Phase Diagrams

The one-, two-, and three-phase areas in the soybean oil/water/phospholipid systems are shown in the phase diagram in Figure 4 which is given as a pseudo-ternary phase diagram. The solubility of the soybean oil in the lamellar phase of the acetone-precipitated phospholipid mixture is less than 10 wt. %. Upon addition of the phospholipid mixture to oil and water the emulsifier precipitates in the form of a lamellar liquid crystalline phase (N), in equilibrium with solutions of SBPap in water (L_1) and in soybean oil (L_2). It is well known that the solubility of PC in water is very low, ca. $1 \times 10^{-10} \text{ M}$ (10). For SBPap, the solubility in soybean oil is $\approx 1 \times 10^{-3} \text{ M}$ (9). This means that practically all of the emulsifier was precipitated in the form of a lamellar phase in the water-oil system. In the presence of charged lipids, the lamellar phase is easily dispersible in the water phase (L_1).

Emulsions

Emulsion stability. Emulsions with varying oil:water ratios were prepared with emulsifier contents from 0.1 to 3 wt. %. In the systems investigated, o/w-emulsions were formed almost exclusively. W/o-emulsions were obtained only with the SBPgr emulsifier when the oil-to-water ratio exceeded 0.6. The dispersing and stabilizing properties of well defined PC and the different phospholipid mixtures were compared in several series of emulsions containing oil-to-water phase in the ratio 0.1 to 0.8. The results are summarized in Figures 5-11, which show the concentration regions in which oil separation, unstable and stable emul-

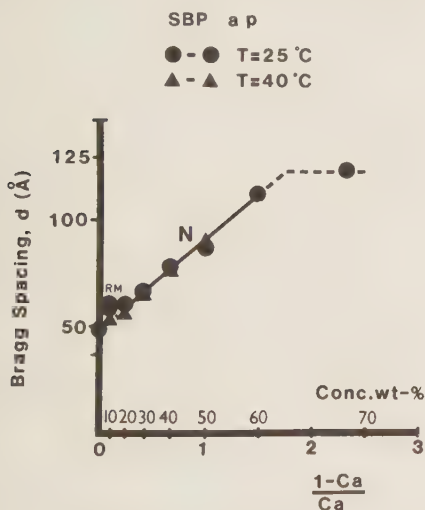


FIG. 2. The swelling behavior of the SBPap in water at (●) 25 and (▲) 40 °C. N = lamellar phase. RM = reversed hexagonal phase.

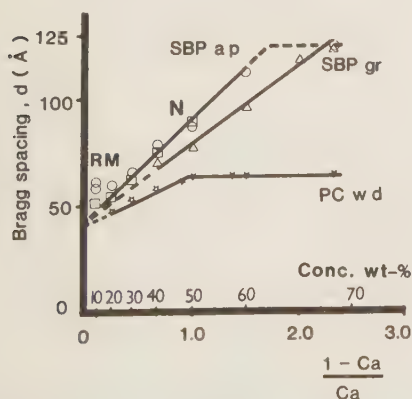


FIG. 3. The swelling behavior of the different soybean phospholipids in water (○) PCwd, (△) SBPgr and (□) SBPap at 25 and 40 °C, respectively. N = lamellar phase, RM = reversed hexagonal phase.

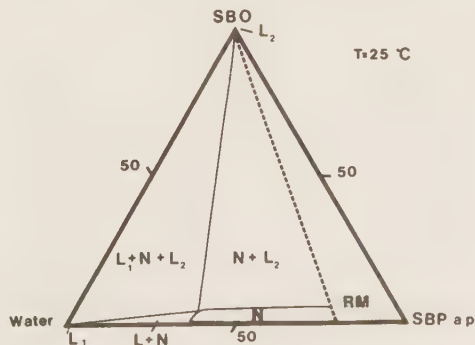


FIG. 4. The phase equilibrium for the pseudo ternary phase-diagram; SBPap-SBO-water at 25 °C. L_1 = water solution of SBPap; L_2 = oil solution of SBPap; N = lamellar phase; RM = reversed hexagonal phase; $L_1 + N$ and $N + L_2$ = two phase regions; $L_1 + N + L_2$ = three phase region.

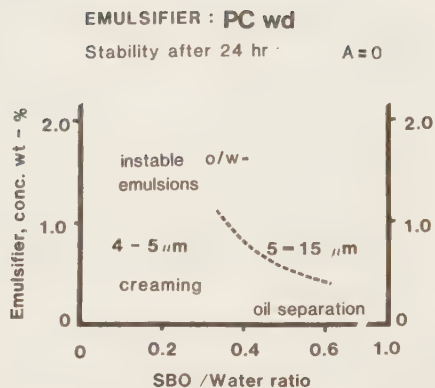


FIG. 5. Emulsions with varying oil-to-water ratios and different concentrations of the emulsifier, PCwd, were investigated within the concentration regions shown in the diagram. The mean globule size and the emulsion stability were determined after 24 hr storage at room temperature.

sions were observed. The mean globule sizes of the emulsions are also given in the diagrams.

The well defined soybean lecithin, PCwd, did not stabilize the o/w-emulsions with respect to creaming and coalescence (Fig. 5). At low emulsifier concentrations, oil separation occurred immediately after the emulsification. The mean globule size was 4-5 μm . Emulsions with a high oil/water phase ratio (0.4 - 0.6) and more than 0.5 wt. % emulsifier were very viscous and did not separate spontaneously within 24 hr. Their large mean globule sizes indicate that these emulsions were mechanically stabilized with respect to creaming while coalescence was not prevented. When the emulsions were stirred or centrifuged at low G-values, oil phase separated immediately.

The acetone-precipitated phospholipid mixture, SBPp, contained 25 mol % of the negatively charged phospholipids PA and PI, i.e., $A = 25$. In this system, a concentra-

tion region with stable o/w-emulsions was observed with a mean globule size varying between 3-5 μm (Fig. 6). At low concentrations of emulsifier and high oil-to-water phase ratios, oil separation occurred immediately after the emulsification. Between these regions there is an intermediate region with creaming and coalescence of oil droplets, the average drop size varying between 5-10 μm . The emulsifier concentration needed to stabilize the emulsions corresponds to ca. 1.25 wt. % of the oil phase.

The granulated phospholipid mixture contained 35 mol % charged lipids. Stable o/w-emulsions were obtained with oil-to-water phase ratios 0.1 to 0.6 and emulsifier concentrations of 0.5 to 2 wt. % (ca. 1.25 wt. % when calculated on the oil phase), the droplet size varying from 3-5 μm (Fig. 7). The mean globule size in the region where creaming occurred was 5-10 μm , which indicates coalescence of the emulsion droplets. The oil phase separated at low emulsifier concentrations and high oil-to-water ratios. When the emulsifier was dispersed in the water phase before emulsification, much more emulsifier was required to stabilize the emulsions (Fig. 8).

Influence of sodium chloride on the emulsion stability. The well-defined soybean lecithin, PCwd, in the presence of sodium chloride, very unstable emulsions were formed. An average droplet size of more than 25 μm indicates rapid coalescence of oil droplets followed by oil phase separation (Fig. 9).

The acetone-precipitated phospholipid mixture, SBPp: more than twice as much emulsifier was required to produce stable emulsions as in salt-free systems (Figs. 10 and 6). In a large concentration region of the system, the electrolyte gave rise to creaming followed by coalescence as shown by the large drop sizes.

The granulated phospholipid mixture, SBPgr: stable emulsions were formed when about twice as much emulsifier was used as in the salt-free system (Figs. 11 and 7). Creaming and coalescence occurred at low emulsifier concentration and a high oil-to-water phase ratio.

Interfacial area and interfacial film thickness. The total o/w interfacial area in the emulsion was calculated from the drop size distribution curves. If it is assumed that all surface-active lipid (the emulsifier) is located at the oil-

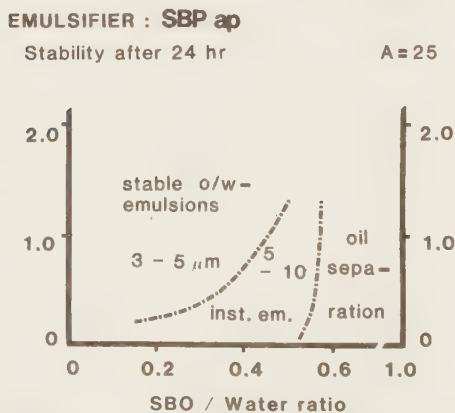


FIG. 6. Emulsions with varying oil-to-water ratios and different concentrations of the emulsifier SBPp were investigated within the concentration regions shown in the diagram. The mean globule size and the emulsion stability were determined after 24 hr storage at room temperature.

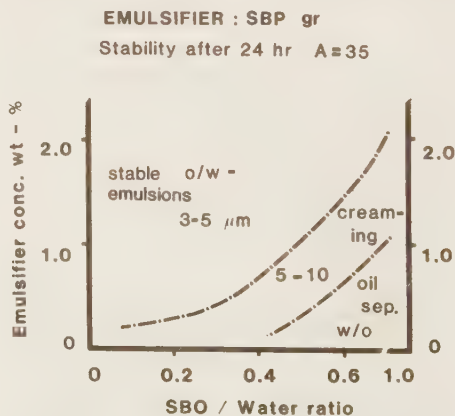


FIG. 7. Emulsions with varying oil-to-water ratios and different concentrations of the emulsifier SBPgr were investigated within the concentration regions shown in the diagram. The mean globule size and the emulsion stability were determined after 24 hr storage at room temperature.

water interfacial area of the emulsion, the thickness of the interfacial phospholipid film may be calculated. Film thicknesses for the different emulsifiers are plotted as a function of the oil-to-water phase ratio in Figures 12-14.

Figure 12 shows that, in spite of the large amounts of emulsifier and thick films, stable emulsions were not obtained with PCwd. SBPap (Fig. 13) stabilizes o/w-emulsions if the interfacial film thickness exceeds ca. 80 Å. At high oil-to-water phase ratios, the amount of emulsifier required to stabilize the o/w-emulsions increases. SBPgr (Fig. 14) also stabilizes o/w-emulsions if the interfacial film thickness exceeds ca. 80 Å. Thicker films are required to stabilize o/w-emulsions with high oil-to-water phase ratios.

The conditions of emulsification in the valve homogenizer, i.e., the shear stress, the interfacial tension and the viscosities of the oil and water phases, respectively, are such that the droplets produced vary between 3-5 μm . Thus, if the emulsifier is able to adsorb at the o/w interface at the moment of emulsification in such a way that the coalescence of droplets is prevented and stable emulsions are formed, the mean globule size will vary between 3-5 μm . The extensive swelling that takes place when the emulsifier contains charged lipids evidently makes such adsorption possible. The minimal amount of lipid that has to be adsorbed can be found by plotting the mean globule size as a function of the interfacial film thickness. Such plots are shown in Figures 15-17. For PCwd (Fig. 15) there is no correlation between amount adsorbed, emulsion stability and mean globule size. However, the diagrams for the mixed emulsifiers, SBPap and SBPgr (Figs. 16 and 17) show that when the concentration of the emulsifiers SBPap and SBPgr is high enough to form interfacial films with a thickness of ca. 80 Å or higher, the mean globule size approaches a minimal mean drop size of 3-5 μm and stable emulsions are formed. It is obvious from Figures 13 and 14 that this correlation is not obtained for emulsions with a high oil-to-water phase ratio.

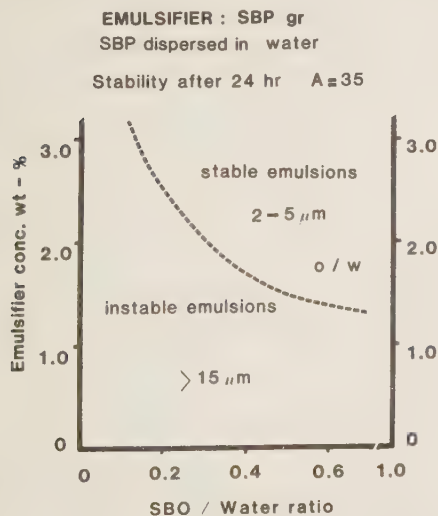


FIG. 8. Emulsions with varying oil-to-water ratios and different concentrations of the emulsifier SBPgr were investigated within the concentration regions shown in the diagram. The mean globule size and the emulsion stability were determined after 24 hr storage at room temperature. The emulsifier was dispersed in water before emulsification.

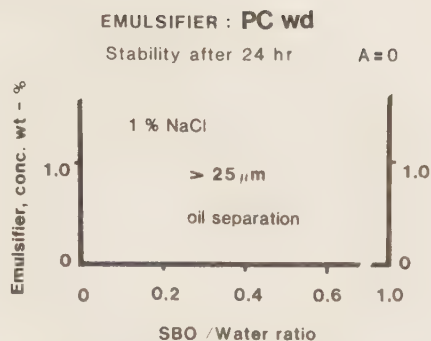


FIG. 9. Emulsions containing 1 wt. % NaCl with varying oil-to-water ratios and different concentrations of the emulsifier PCwd were investigated within the concentration regions shown in the diagram. The mean globule size and the emulsion stability were determined after 24 hr storage at room temperature.

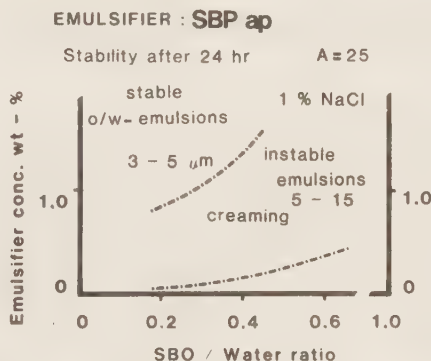


FIG. 10. Emulsions containing 1 wt. % NaCl were investigated at different oil-to-water ratios and emulsifier concentrations of SBPap within the concentration regions shown in the diagram. The mean globule size and the emulsion stability were determined after 24 hr storage at room temperature.

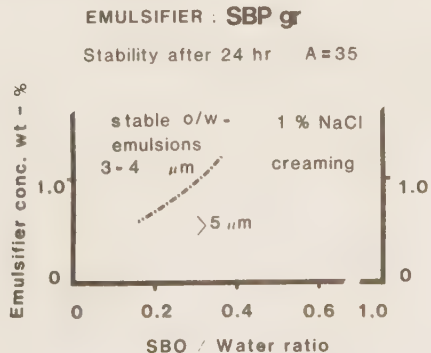


FIG. 11. Emulsions containing 1 wt. % NaCl were investigated at different oil-to-water ratios and emulsifier concentrations of SBPgr within the concentration regions shown in the diagram. The mean globule size and the emulsion stability were determined after 24 hr storage at room temperature.

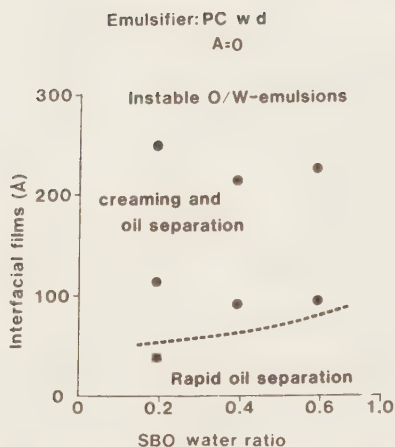


FIG. 12. The thickness of the interfacial films as a function of the oil-to-water ratio for emulsions stabilized with the emulsifier PCwd. (○) Unstable emulsions.

DISCUSSION

It was first demonstrated by Friberg in 1969 that the presence of a lamellar liquid crystalline phase enhanced the stability of o/w-emulsions in the system of *p*-xylene-water-nonionic emulsifier (11). Later, Friberg and coworkers demonstrated that stabilization of o/w-emulsions by means of lyotropic liquid crystals formed by surface active substances could be obtained in different emulsifier systems where these surfactants were used as emulsifiers (12-14). This investigation gives some additional insight into the relationship between liquid crystal formation and emulsion stability.

It should be observed that surface active lipids below their chain melting temperatures (cmt) may form several gel phases that are of great importance for the emulsion

stability at such temperatures (15,16). We have only investigated the emulsion stability above the cmt of the phospholipids and hence no conclusions concerning the relative stability of emulsions stabilized by gel phases as compared to liquid crystalline phases can be drawn from this study.

It is well known that PC swells to a lamellar liquid crystal above its cmt (8). It has been reported that egg PE forms both a lamellar and a hexagonal structure of which the hexagonal packing dominates when the temperature is raised (8). PA swells with water to form a lamellar liquid crystal (17) which probably also is the case for the PI. At pH 6-7, PA and PI are partly dissociated. This introduction of charged groups causes the lamellar phase to incorporate more water (9,18). In the mixed emulsifier consisting of PA, PC, PE and PI only the lamellar packing was found to be simultaneously in equilibrium with the water (L_1) and oil (L_2) solutions. Thus, our results on the swelling behavior of the well defined soybean PC and the mixed phospholipid emulsifiers seem to agree well with the literature data reported for the individual phospholipids. The solubility of the nonpolar triglyceride, i.e., soybean oil, is less than 10 wt. % in the lamellar phase of well defined soybean PC and in the mixed phospholipid emulsifiers (Fig. 4 and Ref. 9) while the solubility of the emulsifier in the water and oil phases is extremely low.

This investigation shows clearly that more than a monolayer of the emulsifier is required to stabilize the o/w-emulsions (Figs. 10 and 11). Since the emulsifiers were dispersed in the oil phase before emulsification it may be assumed that they adsorb at the oil-water interface as soon as the new interface is created. When the amount of emulsifier exceeds a monolayer corresponding to an interfacial film thickness of ca. 20 Å, the excess will precipitate as a lamellar liquid crystalline phase due to the very low monomer solubility in both the water and oil phases. Figures 13 and 14 show that, in the stable emulsion, the film thickness is ca. 80 Å which corresponds to four monolayers. Interfacial films slightly less than 100 Å were earlier found in emulsions stabilized by PC mixed with small amounts of a soap, sodium stearate (9). There are a few points in Figure 17 indicating that even thinner films give stable emulsions. This raises the question of how reliable the judgment of

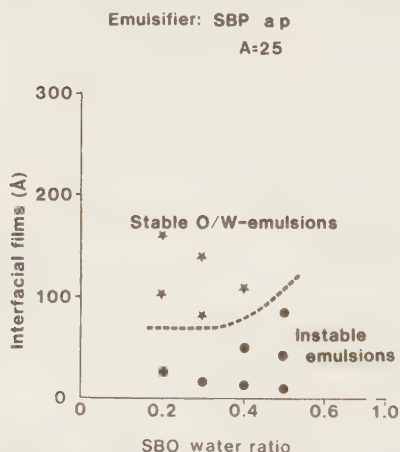


FIG. 13. The thickness of the interfacial films as a function of the oil-to-water ratio for emulsions stabilized with the emulsifier SBP a p. (●) Unstable emulsions; (○) stable emulsions.

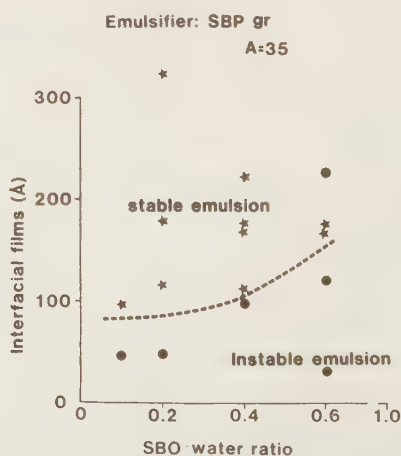


FIG. 14. The thickness of the interfacial films as a function of the oil-to-water ratio for emulsions stabilized with the emulsifier SBP gr. (●) Unstable emulsions; (○) stable emulsions.

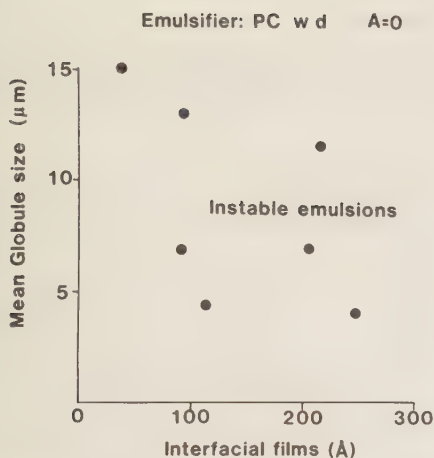


FIG. 15. The mean globule size of emulsions stabilized with PCwd as a function of the interfacial film thickness. (●) Unstable emulsions.

emulsion stability and the calculation of the total interfacial area are.

First of all, it should be noted that in the SBPap and SBPgr, emulsifiers contain 10 and 22 wt. %, respectively, of lipid-soluble material in addition to PA, PC, PE and PI. These compounds are probably surface active and may influence both the swelling behavior and the emulsion stability.

The total interfacial areas have probably been underestimated because the Coulter Counter does not detect particles less than 1 μm. However, only if it is assumed that at least 30-40% of the oil forms smaller droplets varying between 0.1 - 1 μm in size will the interfacial area be large enough to qualitatively change the conclusions. This appears highly unlikely.

Another possibility is that the emulsifier is partly dispersed in either the oil or water phase. Observations between crossed polarizers directly reveal that no birefringent material is present in the oil phase. When the mixed emulsifier SBPgr was dispersed in the water phase much more emulsifier was required to stabilize the emulsions. The emulsifier in this case forms stable dispersions, i.e., liposomes in the water phase which do not adsorb quantitatively at the o/w-interface (Fig. 8). The possibility that such dispersions are also formed in the emulsification process following the dispersion of the lipids in oil cannot be ruled out, although it appears unlikely since the minimal amount of emulsifier required to form stable emulsions is quite sharply defined. Further investigations are required to clarify whether phospholipids dispersed in an oil phase form dispersions of liposomes and emulsion droplet simultaneously.

Our results clearly show that the amount of charged lipids in the emulsifier mixture has a strong influence on the ability of the lamellar liquid crystalline phase to swell with water, i.e., as the fraction of charged lipids increases, a phase with larger and larger interplanar distances will be in equilibrium with the oil and water phases. The formation of these swollen lamellar structures also correlates well with an increased stability of the emulsions formed when the water, oil and liquid crystalline phase is subjected to an emulsifying procedure.

These facts are clearly manifested in the difference

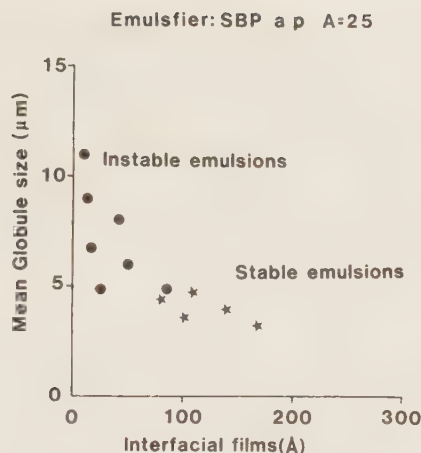


FIG. 16. The mean globule size of emulsions stabilized with SBPap as a function of the interfacial film thickness. (●) Unstable emulsions; (○) stable emulsions.

in behavior between the well defined PC and the mixtures. PCwd is not able to swell extensively with water but addition of a few mol % of sodium stearate causes extensive swelling (9,18). At the same time, the stability of the emulsions formed by this emulsifier, water and soybean oil increases (9). The SBPap and SBPgr both behave similarly to the PCwd containing sodium stearate.

The difference in swelling indicated in Figure 3 probably depends both on the ratios of the different phospholipids PA, PC, PE and PI, and on the presence of minor nonphospholipid compounds. The stabilizing properties of the emulsifiers SBPap and SBPgr are equally good when the emulsions are prepared with distilled water. In the presence of electrolyte, slightly more of the SBPap with $A = 25$ is required to stabilize emulsions as compared to the SBPgr

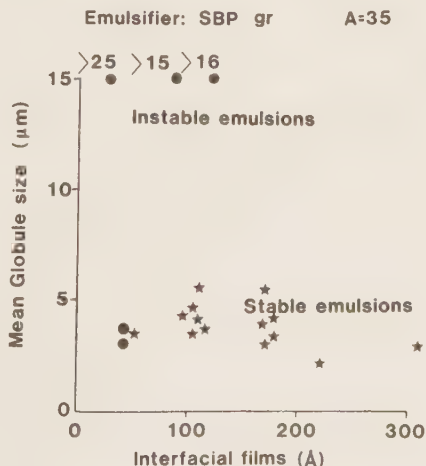


FIG. 17. The mean globule size of emulsions stabilized with SBPgr as a function of the interfacial film thickness. (●) Unstable emulsions; (○) stable emulsions.

with $A = 35$ (Figs. 10 and 11). According to the theory of colloidal stability, there are two types of forces which influence the coalescence (coagulation) of dispersed emulsion droplets, i.e., (a) the London-van der Waals attraction forces and (b) the electrostatic repulsion forces between electrical double layers. As these forces operate independently, the total interaction energy is obtained by summation. If the surface potential is reduced or the ionic strength is increased, the theory predicts that the potential energy barrier against coalescence is lowered (19). The decrease in stability against coalescence when salt is added indicates that the electrostatic repulsion forces originating from the amount of charged lipids in the emulsifier play an important role for the emulsion stability.

In the emulsions stabilized with the well defined PC, creaming and oil separation were observed (Fig. 5) whereas limited concentration regions with stable emulsions could be obtained with the mixed phospholipid emulsifiers (Figs. 6-11). These emulsifiers seem to adsorb more easily at the oil-water interface than the well-defined phospholipid (Figs. 12-14). One explanation may be that the charged PA and PI in the phospholipid mixtures are more soluble in water; hence, the diffusion to the oil-water interface is faster as compared to the electrically neutral substances.

Friberg has suggested that the presence of a multilayer structure on the surface of the oil droplets reduces the Van der Waals attraction forces between the dispersed oil globules (14). As a consequence of this the force, the tendency to coalescence will be reduced. In addition to the mechanism suggested by Friberg et al., our investigation demonstrates that the repulsion forces occurring from the charged molecules in the emulsifier also contribute to the prevention of coalescence; the droplet stability is greatly enhanced if lamellar liquid crystals are able to incorporate large amounts of water due to the presence of charged lipids which can be adsorbed at the o/w-interface.

ACKNOWLEDGMENTS

A. Biverstedt and T. Gabrán characterized the phospholipids. A.-M. Wennerberg and I. Larsson prepared the emulsions. P. Stenius provided valuable comments on this manuscript. The Swedish Board for Technical Development provided a grant for this project.

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[Received March 2, 1981]

**PHASE EQUILIBRIA IN THE SYSTEM DIMYRISTOYLPHOSPHATIDYL
CHOLINE-N-HEXADECYL-N, N, N-TRIMETHYLAMMONIUM BROMIDE
AND WATER AT 30°C. SWELLING BEHAVIOR OF THE LAMELLAR PHASE
WITH DIFFERENT ELECTROLYTE SOLUTIONS**

Lisbeth Rydhag and Thomas Gabrán

The Institute for Surface Chemistry,
Box 5607, S-114 86 Stockholm, Sweden

ABSTRACT

The phase equilibria of a swelling amphiphile, dimyristoylphosphatidylcholine (DMPC) and a cationic soap, cetyltrimethylammoniumbromide (CTAB) has been investigated in water at 30°C. The swelling of the lamellar phase of DMPC in water increases dramatically in the presence of a few mole-% of CTAB. Maximum swelling of the lamellar phase in water is obtained when the lamellar phase is in equilibrium with aqueous solution whose concentration of CTAB is less than the critical micelle concentration (*cmc*). Vesicles are easily formed by the lamellar phase in this region. When the *cmc* of CTAB is exceeded DMPC is solubilized in micelles. The solubilizing capacity corresponds to about 1 mole of DMPC per 3 moles of CTAB. The phase behaviour of DMPC and CTAB in water is very similar to the corresponding equilibria for amphiphile compounds with shorter hydrocarbon chains.

The swelling of DMPC and CTAB is influenced by the presence of electrolytes in the water phase. Due to electrostatic effects the swelling of the lamellar phase is lower in electrolyte solutions than in pure water.

INTRODUCTION

It was shown more than a decade ago that the maximum swelling capacity of water-dispersible amphiphilic lipids is substantially increased by the addition of small amounts of a long-chain ionic surfactant (1). We have recently shown that the maximum swelling of lipid mixtures also can be correlated with the amount of charged lipids in the mixture (2, 3). Few such studies, however, have been extended to a full investigation of the phase equilibria in systems consisting of water and lipid mixtures. In this paper we show that a typical system consisting of a swelling lipid amphiphile, an ionic surfactant and water in many respects is similar to systems consisting of an ionic surfactant, a simple nonionic amphiphilic compound (e.g. a fatty acid at $\text{pH} < \text{pK}_a$ or an aliphatic alcohol) and water. For the latter systems, the phase equilibria have been investigated in much greater detail (4, 5). The differences between the systems containing typical swelling amphiphiles and those containing nonionic amphiphiles with shorter chains appear to be a consequence of the extremely low solubility and the higher melting point of the former compounds.

The phase equilibria of water and a swelling amphiphile combined with an electrically neutral zwitterionic surfactant (6) on an ionic surfactant such as bile salts (7) have been determined earlier. In the latter system the formation of monodisperse liposomes has been observed (7) and several papers have dealt with the preparation of monodisperse vesicles by dilution of mixed micellar solutions of the swelling amphiphile and the bile salt with water or buffer solution (8-10).

Several investigations deal with the interactions of micelle-forming surfactants with vesicles which were prepared from swollen dispersible amphiphiles. Fu and Laughlin (11) studied the interactions of homologues of 6-alkylammoniumhexanoates with unilamellar distearoyl phosphatidylcholine (DSPC) vesicles. The vesicles were destabilized when the concentration of the zwitterionic surfactant exceeded the critical micelle concentration (c_{mc}). Several other studies of the interaction of anionic, cationic or nonionic surfactants with vesicles or membranes

components have been published (12-15)¹.

Kunieda and Shinoda (16) have investigated the phase behaviour of dioctadecyldimethylammonium chloride, octadecyltrimethylammonium chloride and water. The former salt behaves as a swelling amphiphile although it is not nonionic; the investigation does illustrate the main features of the phase behaviour of a swelling amphiphile and a water-soluble surfactant.

In our opinion, a proper understanding of the factors that affect the swelling of the amphiphiles and their dispersibility as vesicles or liposomes can only be obtained with reference to a proper knowledge of relevant thermodynamic phase equilibria. This opinion is based on the considerable insight into the behaviour of surfactants in general which has emerged from studies of the phase equilibria (2, 5, 17-21). Surfactants, whether they are anionic, nonionic, cationic or zwitterionic, all associate in water or swell with water to form certain phases such as micellar solutions and liquid crystalline structures (4, 5, 21, 28). The knowledge of the phase equilibria makes the choice of surfactants for improved foam and emulsion stability easier (2, 17-21). In this paper we report on studies of the phases formed in the system dimyristoylphosphatidylcholine (DMPC)-cetyltrimethylammoniumbromide (CTAB)-water. Considerable precautions were taken to ensure work with compounds under reproducible conditions. Thus a lipid with saturated hydrocarbon chains was chosen to make extensive precautions against oxidation unnecessary and the cationic surfactant was chosen to avoid the use of preservatives.

From the knowledge of the phase equilibria of DMPC and CTAB in water it is possible to predict in which concentration regions liposomes might be prepared. In subsequent papers we will report studies of vesicle stabilities in this three-component system (27) and emulsion stabilities in a system consisting of the three components and an oil. The studies have so far been limited to phases which are in equilibrium with aqueous solutions of DMPC + CTAB.

¹) This reference list is not intended to be exhaustive.

MATERIALS AND METHODS

Materials

The dimyristoyl-L- α -phosphatidyl choline (DMPC) was from Sigma Chemical Co., USA. It was shown by gas chromatographic analysis of the fatty acid methyl esters (22) that 99.9% of the hydrocarbon was saturated C₁₄ chains. Thin layer chromatography was used to check the absence of polar and non-polar impurities (23). The chain melting temperature (*CMT*) was determined by differential thermal analysis on a Mettler TA 2000 instrument. It was found to be (296.7 ± 0.5) K which is in agreement with previous data on *CMT* of DMPC (24, 29).

N-hexadecyl-N,N,N-trimethyl ammonium bromide (cetylmethylammoniumbromide, CTAB) was analytical grade from Merck Ag, Darmstadt, GDR. Conductivity measurements were used to determine the Krafft point in water which was found to be 300 K.

The salts were all of analytical grade. The water was triply distilled with a conductivity of about $0.5 \mu\text{S cm}^{-1}$.

X-ray diffraction

X-ray diffraction patterns were recorded with a Kiessig low angle X-ray scattering camera with point collimation (R. Seifert Co., GDR). The camera was equipped with a thermostatted sample holder ($\pm 0.02^\circ\text{C}$). The distance between the specimen and the film was 500 mm. All exposures were made using Ni filtered Cu _{α} -irradiation on Ilford Industrial G₁ or Kodarex C film. The time of exposure varied from 20 h to one week.

Determination of phase equilibria and identification of phases

All phase equilibria were determined at 303 K (30°C). This temperature was chosen because it is well above the *CMT* of the DMPC and the Krafft point of the CTAB.

The following procedure was used to ensure attainment of equilibrium in the multiphase systems. The DMPC and the CTAB were dissolved together in trichloromethane which was then evaporated under reduced pressure. Water was then added and the

systems were equilibrated at $(30 \pm 1)^{\circ}\text{C}$ for at least six days. It was checked by thin layer chromatography that hydrolysis of DMPC did not occur during the equilibration procedure.

The phase was separated by ultracentrifugation at $(30 \pm 1)^{\circ}\text{C}$ for at least 17 h at 60000 $\times g$, using a Beckman Model L2-65B preparative ultracentrifuge. Samples of the separated phase were analyzed quantitatively for water, DMPC and CTAB. The structure of the liquid crystalline phases was inferred from their appearance between crossed polarizers in the microscope and confirmed by X-ray diffraction measurements. Details of this identification procedure are given in ref. (25).

The amount of water was determined by weighing before and after the following drying procedure. The samples were first dried under reduced pressure for two days in an oven at 55°C and then for 20 h (or until constant weight) at 30°C in a vacuum drier. The amount of DMPC was determined by analysis of the phosphorous according to Lowry and Tinsley (26). The CTAB was analyzed using ^{14}C -labelled CTAB and liquid scintillation measurements in a Packard Tri Carb 3375 instrument.

Preparation and characterization of unilamellar vesicles

Unilamellar vesicles were prepared from samples whose total composition was located in the two-phase region in which lamellar phase and an aqueous monomeric solution of DMPC and CTAB ($L_1 + D_1$) are in equilibrium (see Fig. 1). The compositions were chosen in such a way that the concentration of CTAB in the L_1 phase was below the *cmc*. Details of the preparation and characterization of the vesicles will be given in a subsequent paper. A preliminary report was given in ref. (27).

RESULTS AND DISCUSSION

Phase equilibria

Description of the phase diagram. The general appearance of the phase diagram for DMPC/CTAB/water in regions with more than ca 40% water was determined by microscopic and visual examination supplemented by X-ray diffraction measurements. About 300 samples in one- and two-phase regions were examined. The phase diagram is given in Fig. 1.

The DMPC swells with water to a liquid crystalline phase which is characterized by Bragg spacings in the ratio 1:1/2:1/3 ... (29, 38). This is characteristic of a lamellar phase (D) or (L_α) in the nomenclature of Luzzati (28). Fig. 2a shows the repeat distance, d , of the phase as a function of ϕ_a^{-1} (ϕ = volume fraction of DMPC). As expected for a lamellar phase (28, 29), the d -values increase linearly with ϕ_a^{-1} up to 40 weight-% of water, which is the maximum amount of water that can be incorporated in the phase (29, 38). For higher amounts of water, d remains constant since the lamellar phase is in equilibrium with a very dilute solution of DMPC in water.

When CTAB is added the ability of the lamellar phase to incorporate water increases very rapidly. This dramatic increase is shown clearly in Fig. 2. The effect is so large that the phase boundary cannot be determined from X-ray determinations even when only 1% of CTAB is added (when the concentration of water in the phase exceeds 80% the intensity of the diffracted X-ray becomes too weak to be detected even with prolonged exposure times.) The ability to incorporate water increases up to a weight ratio $r = w(\text{CTAB}) / [w(\text{CTAB}) + w(\text{DMPC})] \approx 0.5$. For higher r , the system first, for a narrow range of r values divides into a much less swollen lamellar phase and a micellar solution L_1 and then, for even higher r , forms a micellar solution of CTAB which solubilizes DMPC.

In the binary system CTAB/water a liquid crystalline phase consisting of hexagonally packed infinitely long rods of the surfactant in an aqueous environment (hexagonal phase E) is observed in equilibrium with saturated micellar aqueous solution (30). This phase probably is able to incorporate some

DMPC. Since we are, above all, interested in those parts of the phase diagram in which vesicle formation usually occurs we have only studied the region of the phase diagram which is to the left of the dashed line in Fig. 1 in detail.

The two-phase region $L_1 + D$ for $r < ca\ 0.5$. The phase boundaries in the two-phase region between the strongly swelling lamellar phase and dilute aqueous solution was determined for a series of samples with the compositions shown in Fig. 3. The equilibrated samples were viscous, slightly turbid and contained birefringent material. For $r < 0.2$ it was possible to separate these samples into lamellar phase and aqueous solution by ultracentrifugation. The results of chemical analysis of the two phases are shown in Fig. 3. These analyses all show that the swollen lamellar phase is in equilibrium with aqueous solutions whose concentration of CTAB is less than the *cmc*.

The solubility of DMPC in pure water was found to be 0.004% or $6 \cdot 10^{-5}$ mole dm^{-3} . A much lower solubility (10^{-10} mole dm^{-3}) has been reported for egg phosphatidyl choline (31). The solubilities of DMPC in solutions of CTAB below the *cmc* are higher but the experimental values are scattered (Fig. 4). Dynamic light scattering in these solutions showed that they contained particles with a diameter of ≈ 200 nm. This is probably due to incomplete separation of the lamellar phase from the solutions. Vesicles are, in fact, very easily formed by the lamellar phase in this region (27). When the *cmc* of CTAB is exceeded, DMPC is solubilized in micelles and the solubility increases rapidly.

Maximum incorporation of water in the lamellar phase occurs for $0.3 < r < 0.4$ (Fig. 3), i.e. 30-40 weight-% CTAB in the lipid mixture. Separation of lamellar phase from the aqueous solution in this region was virtually impossible, obviously due to the extremely small density difference. Several equilibrated samples were ultracentrifuged and the top and bottom layers in the centrifuged samples were analyzed. In this way it was shown that for molar ratios CTAB/DMPC between 0.8 and 1.2 which correspond to 30 to 40 wt% of CTAB in CTAB + DMPC, it was impossible to separate samples containing 99% water into $L_1 + D$, i.e. they still appear to be homogeneous lamellar phase.

If we assume that the lamellar phase swells linearly with

water we may calculate the repeat distance of the lamellar layers. This is shown in Fig. 5 as a function of the molar ratio CTAB/DMPC. We emphasize that due to low scattering intensities only some of these values have been verified directly by X-ray measurements.

The d values are very large, but by no means unreasonably so except, perhaps, for the very largest contents of water. Interlamellar distances of this size have, for example, been measured repeatedly for electrostatically stabilized foam films and for swollen montmorillonites.

Fig. 6 shows the distribution of CTAB between the aqueous solution and the lamellar phase. The *total* concentration of CTAB in the system is higher than the *cmc* for all the points given in Fig. 6, but CTAB is enriched in the lamellar phase in such a way that the equilibrium concentration in the L_1 -phase is far below the *cmc*. Thus, at these concentrations the lamellar structures formed by DMPC and CTAB is an energetically more favourable environment for CTAB than would be a spherical micelle. When the equilibrium in L_1 -phase is above the *cmc*, CTAB is not preferentially distributed into the lamellar phase (Fig. 6).

It is reasonable to assume that virtually all of the CTAB in the lamellar phase is built into the lamellae with the cationic end group located in the lamellar surface and with molecular cross-sectional areas as inferred from previous X-ray diffraction measurements of CTAB-n-hexanol-water. The cross-sectional areas of CTAB in the lamellar phase varied between $0.25\text{--}0.30\text{ nm}^2$ (30). A molar ratio CTAB/DMPC between 0.8 and 1.2 then corresponds to $(1.0 - 1.2) \cdot 10^{14}$ unit charges per cm^2 or a surface charge density of $16\text{--}19\text{ }\mu\text{C}/\text{cm}^2$. This very high surface charge density in combination with the low solubility of DMPC in water may account for the very drastic swelling of the lamellar phase. Quantitative calculations of the electrostatic repulsion forces and their importance for the prediction of the phase boundaries in binary and ternary systems of surfactant, water and nonionic amphiphile have very recently been described by Wennerström *et al.* (32, 33). An application of the theory to the system DMPC/CTAB/water will be published elsewhere.

The two-phase region $D + L_1$ for $r > 0.5$ and micellar L_1 solutions. Fig. 3 shows that as the concentration of CTAB in

the D phase increases above $r \approx 0.5$ the concentration of CTAB in the equilibrium L_1 solution exceeds the *cmc* of CTAB. This results in solubilization of the DMPC into the micelles. The solubilized amount increases linearly with the concentration of micellar CTAB and corresponds to a solubilizing capacity of about 1 mole DMPC per 3 moles of CTAB.

As a consequence of the formation of the energetically more favourable mixed micelles in aqueous solution, the lamellar phase is not stable for r values exceeding 0.5. The two-phase region $L_1 + D$ where L_1 is a micellar solution is very narrow. In practice, it is impossible to achieve complete separation of the two phases in this region. Similar difficulties have been noted in systems of amphiphiles with much shorter hydrocarbon chains and it has been suggested that other liquid crystalline phases may be formed in the $D + L_1$ region. However, these suggestions have not been verified. In recent experimental studies it is concluded that, for reasons which are not completely clear, the phases L_1 and D are very difficult to separate in this region (34), but no other phases are formed.

Equilibria at lower concentrations of water. It was already mentioned that in the binary system water/CTAB a hexagonal phase of type E is formed when the solubility of CTAB in water is exceeded. At even lower water contents a lamellar phase of type D appears. It was shown by Ekwall *et al.* (30) for the system CTAB/hexanol/water that this D -phase is able to incorporate hexanol in such a way that it forms a continuous D -phase region which extends towards very high concentrations of water much in the same way as the D -phase region shown in Fig. 3. It is conceivable that the CTAB/DMPC/water equilibria behave in the same way, but we have not investigated this. Nevertheless, the similarity between the phase behaviour shown in Figs. 1 and 3 and the corresponding equilibria for amphiphilic compounds with shorter hydrocarbon chains is striking. For short-chain non-ionic amphiphiles like alcohols and fatty acids there will be an organic solution region at high concentration of the amphiphile, while long-chain compounds just swell with water to form liquid crystalline phases.

The swelling of the lamellar phase in water, Tris-HCl buffer and NaCl solutions

The volume fraction of lipid (i.e., CTAB + DMPC), ϕ_a is calculated from (35)

$$\phi_a = \frac{V_a}{V_a + V_w} = \left[1 + \frac{v_w \cdot c_w}{v_a \cdot c_a} \right]^{-1}, \quad (1)$$

where V_w , v_w and c_w are the total volume, partial specific volume and weight concentration, respectively, of water and V_a , v_a and c_a are the corresponding quantities for the lipid mixture (CTAB + DMPC).

Provided that none of the water is incorporated in the lipid bilayer, the lamellar geometry implies that

$$d_a = d \cdot \phi_a, \quad (2)$$

where d_a is the thickness of the lipid bilayer and d the lamellar repeat distance which is given directly by the primary Bragg spacing in the X-ray diffractograms. Knowing d and ϕ_a , the specific area per polar head group may be calculated from

$$S = \frac{2}{N_s}, \quad (3)$$

$$N_s = \frac{\phi_a d N_A}{M \cdot v_a}, \quad (4)$$

where N_s = the number of lipid molecules (CTAB + DMPC) per unit surface of one lamella, N_A = Avogadro's number and M = the mean molecular weight of the lipid mixture.

If d_a is independent of ϕ_a a plot of $\log d$ versus $-\log \phi_a$, according to eq. (2), should give a straight line with slope 1. If d_a or the partial molar volumes are independent of ϕ_a or change linearly with ϕ_a , a plot of d versus ϕ_a^{-1} will be linear; in either case extrapolation to $\phi_a = 0$ gives a measure of the thickness of the lipid bilayer without any water.

If the lamellar phase swells in such a way that d_a is affected by ϕ_a (i.e. the swelling is not simply one-dimensional) it is not possible to distinguish between the contributions of d_a and the thickness of the water layer to d . Such cases will give a slope different from 1 in the $(\log d / -\log \phi_a)$ plot, or

non-linearity.

These simple relationships have been used to investigate the swelling of the lamellar phase in some detail.

For the partial specific volume of DMPC the value $0.939 \text{ cm}^3/\text{g}$ reported in ref. (36) has been used both for DMPC alone and for the DMPC/CTAB mixtures. For the partial specific volumes of water, tris-HCl buffer and NaCl solutions we have used the corresponding volumes for the pure solvents.

DMPC/CTAB/water. The swelling of the lamellar phase in this system is shown in Fig. 2. The extrapolated values of d_a are given in Table I, and the slopes of $\log d$ versus $-\log \phi_a$ in Fig. 7. The extrapolated values do not vary very systematically with the amount of CTAB but are in the range expected for two extended C_{14} chains (4 nm) in the liquid crystalline phase. The swelling, however, is clearly not one-dimensional until about 6 mole-% CTAB is added, but then (within experimental error) the slopes are ≈ 1 up to 40% CTAB.

This will also influence the surface areas per head group which is the inverse of d_a , see eqs. 3 and 4. The variation of S as a function of ϕ_a^{-1} is shown in Fig. 8.

DMPC/CTAB/Tris-HCl buffer. Preparation of liposomes and studies of the swelling of lipids with water often are made in buffer solutions. We have investigated the swelling in a typical buffer solution, 10 mmole/dm^3 tris-hydroxymethylaminomethane-hydrochloric acid (Tris-HCl). The Bragg spacings as functions of ϕ_a^{-1} are summarized in Fig. 9 and the extrapolated values of d_a are given in Table 1. These are larger than the values found for swelling with water, and as Fig. 3 shows, more CTAB ($r > 0.098$) has to be added before ideal one-dimensional swelling is obtained. The value of d_a (4.9 nm) obtained for DMPC with 5.1 weight-% CTAB is too large to be reconcilable with a lamellar bilayer which indicates that the extrapolation is not applicable, i.e. the phase does not swell one-dimensionally.

DMPC swells to a buffer solution content of 46 weight-%. This swelling is greatly enhanced by the addition of 2.6% CTAB (see the curve for $r = 0.026$ in Fig. 9) but in contrast to the swelling in water, the swelling stops at lipid concentrations which can still be detected by X-ray. Addition of 9.8 and

16.2% CTAB extends the swelling to limits which cannot be detected in this way.

The non-linear swelling for $r < 0.098$ is also reflected in the mean areas per head group (see Fig. 10). For pure DMPC S increases with increasing buffer concentrations and reaches a maximum of 0.63 nm^2 . With 2.6 and 5.1 weight-% of CTAB S still varies with ϕ_a , but for $r > 0.98$ S is approximately constant at $0.61\text{--}0.62 \text{ nm}^2$.

DMPC/CTAB/NaCl solution. To compare with the swelling in the buffer solution, the swelling was also investigated for 5 mmole/dm^3 NaCl, which has the same ionic strength as the buffer solution.

Fig. 11 shows the Bragg spacings versus ϕ_a^{-1} and Fig. 12 the mean areas per polar end group. For pure DMPC, 45% NaCl solution can be incorporated by swelling. Addition of 5% CTAB to the DMPC causes swelling to more than 75% with the NaCl solution. The DMPC clearly does not swell linearly (see Fig. 3) but addition of 5% CTAB gives nearly ideal swelling. The extrapolated d_a -value for DMPC is too large (4.7 nm) and S increases with increasing ϕ_a . For the DMPC/CTAB mixture S is almost constant and d_a has a reasonable value (Table 1). The behaviour is quite similar to the DMPC/CTAB/water system with $r = 0.05$ (Figs. 2 and 4).

SUMMARY

The presence of only a few percent of CTAB dramatically enhances the swelling of the lamellar phase, whether with water, Tris/HCl buffer or NaCl solution. The swelling is lower in electrolyte solutions than in pure water and, thus, primarily is due to electrostatic effects (37). With Tris/HCl the swelling, however, is also non-linear up to much higher CTAB concentrations than with water or NaCl, which indicates specific interactions between the trishydroxymethylaminomethane ion and the end groups of DMPC. Pure DMPC does not swell linearly with any of the solvents. It should be noted that the very rapid increase in swelling with even small amounts of charged lipid may explain variations in results obtained with lecithins of different origin.

ACKNOWLEDGEMENT

Professor Per Stenius is gratefully acknowledged for valuable comments on this manuscript. The project was financed by a grant from the Swedish Board for Technical Development.

Legends to Figures

- Figure 1 Phase diagram at 30°C for the system water/dimyristoylphosphatidyl choline (DMPC)/cetyltrimethylammoniumbromide (CTAB). The diagram is incomplete for concentration ranges to the right of the dashed line. The hexagonal phase *E* indicated on the binary axis H₂O/CTAB probably is able to incorporate DMPC, but the phase boundaries are not known in detail.
- Figure 2 Repeat distance d of the lamellar phase as a function of ϕ_a^{-1} (ϕ_a = volume fraction of DMPC or DMPC + CTAB) for different weight ratios. $r = w[\text{CTAB}] / w[\text{CTAB} + (\text{DMPC})]$. (a) $r = 0 - 0.1$. Note: The ordinate scale of each line has been shifted 2 nm with respect to the one below. (b) $r = 0.304, 0.402$. No shifts in the scale of the ordinate. Dashed line: extrapolated boundary for pure DMPC. $\star-\star$ $r = 0$, $\blacktriangle-\blacktriangle$ $r = 0.01$, $\blacksquare-\blacksquare$ $r = 0.02$, $\circ-\circ$ $r = 0.03$, $\star-\star$ $r = 0.058$, $\emptyset-\emptyset$ $r = 0.10$, $+ - +$ $r = 0.302$, $\ominus - \ominus$ $r = 0.402$.
- Figure 3 Expanded detail of the lower left-hand corner in Fig. 1. The right-hand leg of the triangle gives the weight ratio r . The sample compositions from which phase boundaries were determined are given as dots on the tie-lines. Dashed lines: estimated boundaries (incomplete or impossible separation of phases).
- Figure 4 Concentration of DMPC in CTAB solutions saturated with DMPC. The dashed line indicates the *cmc* of CTAB.
- Figure 5 Estimated repeat distances of the lamellar phase as a function of the molar ratio CTAB/DMPC and the weight-% of CTAB in CTAB + DMPC. For molar ratios < 0.8 or at < 30 wt-% the points are based on analysis of separated samples of $L_1 + D$. Between molar ratios of 0.8 and 1.25 the samples appeared homogeneous even with 99% of water.

Figure 6

Concentration of CTAB in the lamellar phase as a function of the equilibrium concentration of CTAB in the aqueous solution L_1 . Dashed line: *cmc* of CTAB.

Figure 7

The slopes of plots of $\log d$ versus $-\log \phi_a$ for the lamellar phase formed by CTAB + DMPC in water, O-O Tris-HCl buffer, ☆-☆ and 5 mmole/dm³ NaCl solution, □-□ .

Figure 8

Mean surface areas per head group in the lipid bilayers as functions of ϕ_a^{-1} . Note that separate scales are used for each r . ☆-☆ $r = 0$,
 ▲-▲ $r = 0.01$, ■-■ $r = 0.02$, O-O $r = 0.03$,
 ★-★ $r = 0.058$, ∅-∅ $r = 0.10$, +-+ $r = 0.302$,
 Θ-Θ $r = 0.402$.

Figure 9

Repeat distance d of the lamellar phase as a function of $\bar{a}^{-1}$ for different r , swelling in 5 mM Tris-HCl buffer. The ordinate scale of each line has been shifted 2 nm with respect to the one below. ☆-☆ $r = 0$, O-O $r = 0.026$, ◆-◆ $r = 0.051$, ∅-∅ $r = 0.098$, ★-★ $r = 0.162$.

Figure 10

Mean surface areas per head group in the lipid bilayers as functions of ϕ_a^{-1} , swelling in 5 mM Tris-HCl buffer. ☆-☆ $r = 0$, ■-■ $r = 0.026$, O-O $r = 0.051$, ★-★ $r = 0.098$, ∅-∅ $r = 0.162$.

Figure 11

Repeat distance d versus ϕ_a^{-1} for CTAB/DMPC in 5 mmole/dm³ NaCl, ☆-☆ $r = 0$, ★-★ $r = 0.05$.

Figure 12

Mean areas per head group for DMPC/CTAB in 5 mmole/dm³ NaCl as function of ϕ_a^{-1} . ☆-☆ $r = 0$, ★-★ $r = 0.05$.

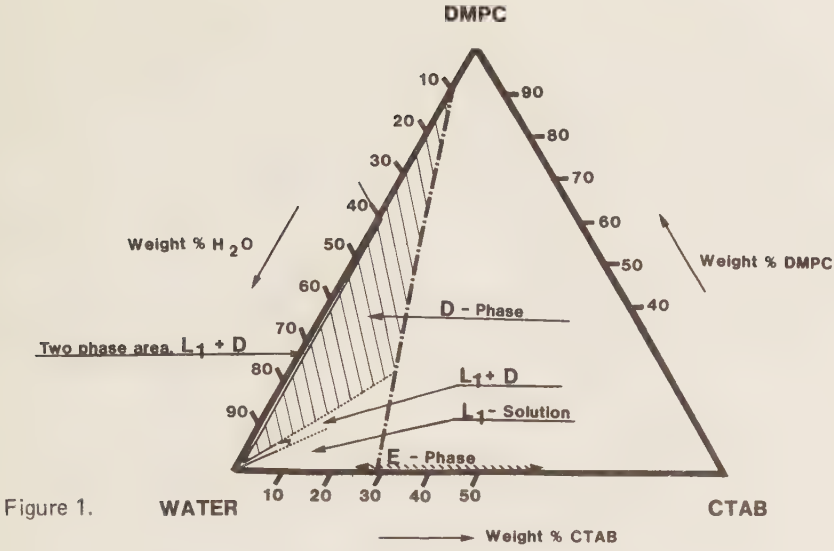


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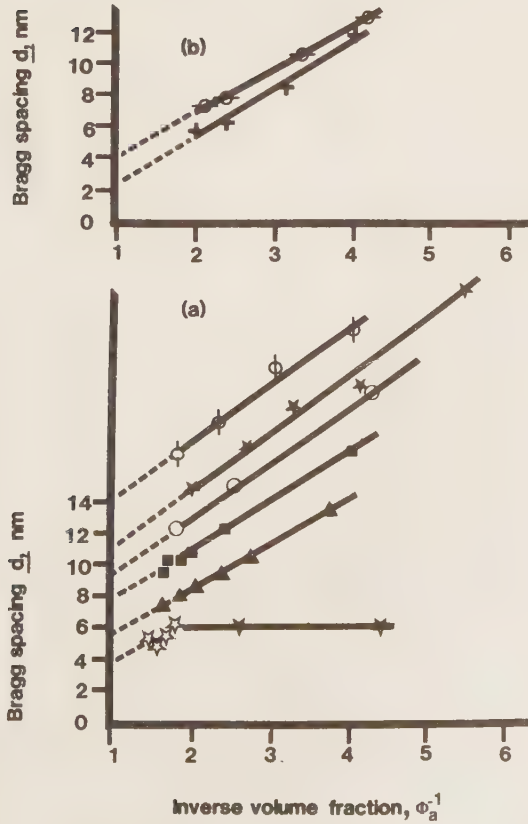


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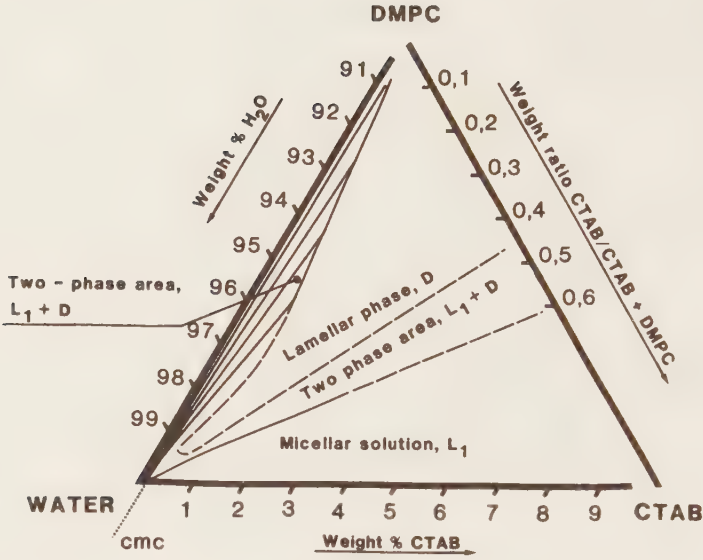


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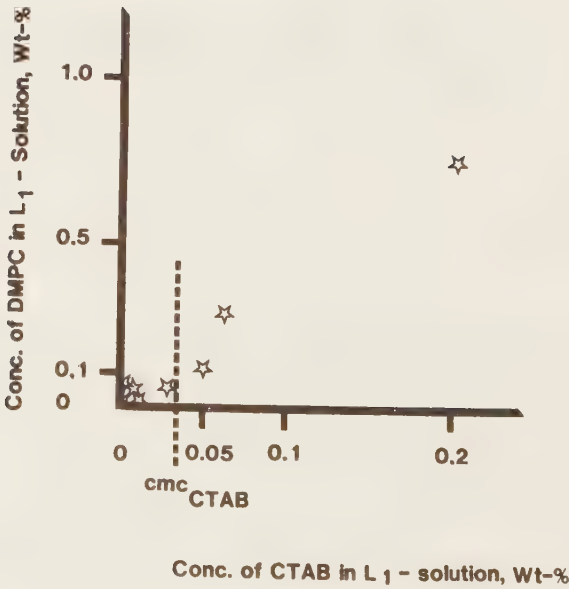


Figure 4.

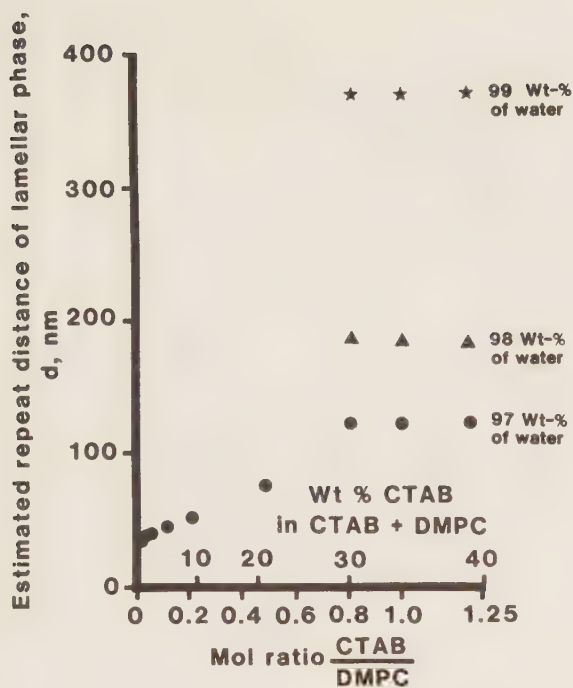


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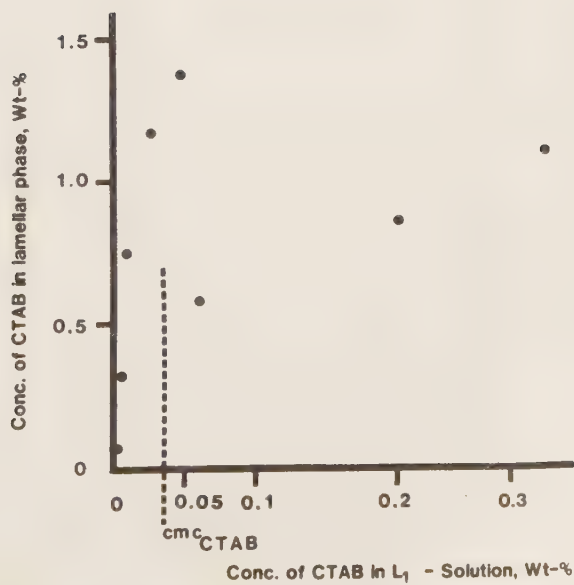


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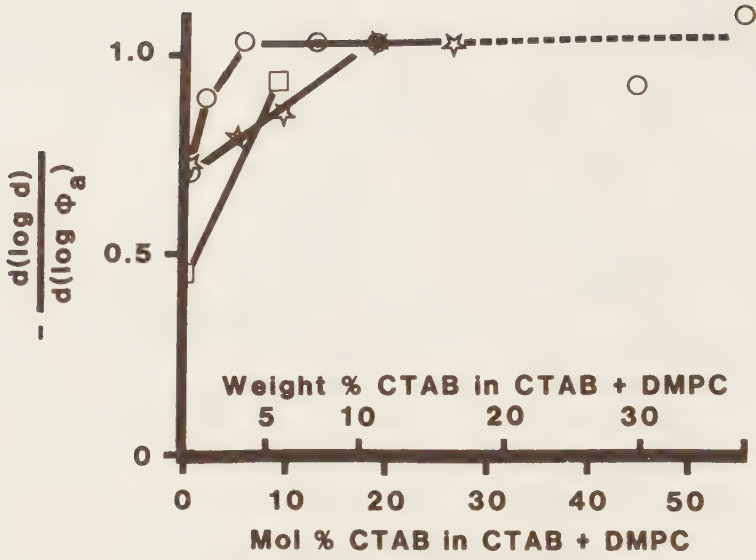


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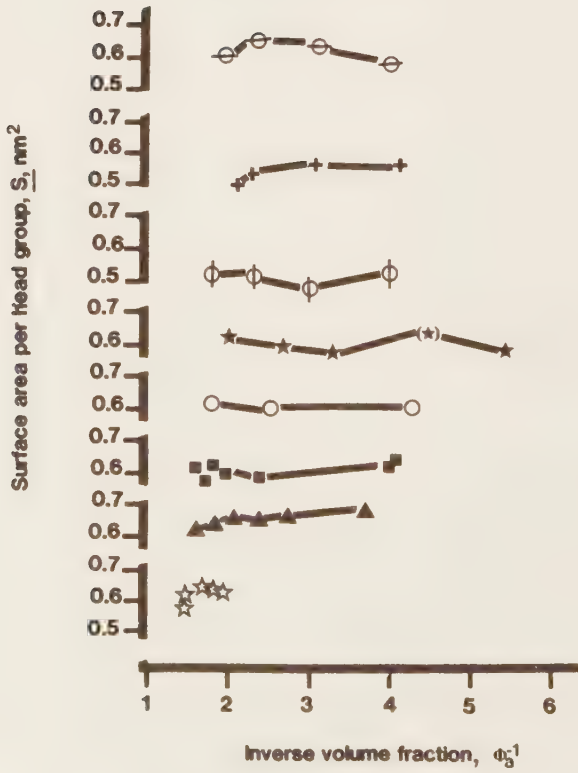


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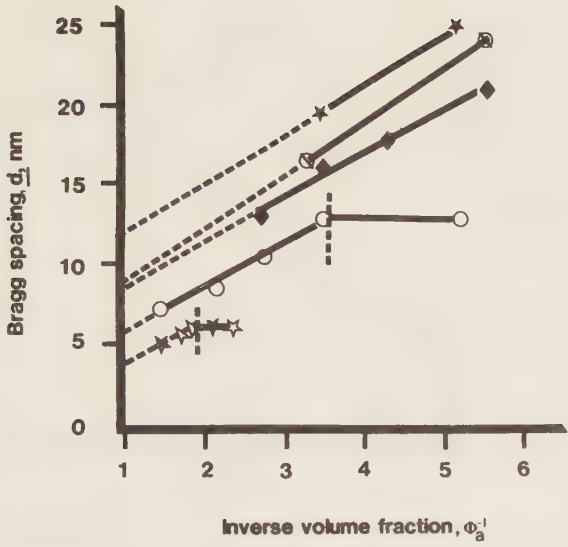


Figure 9.

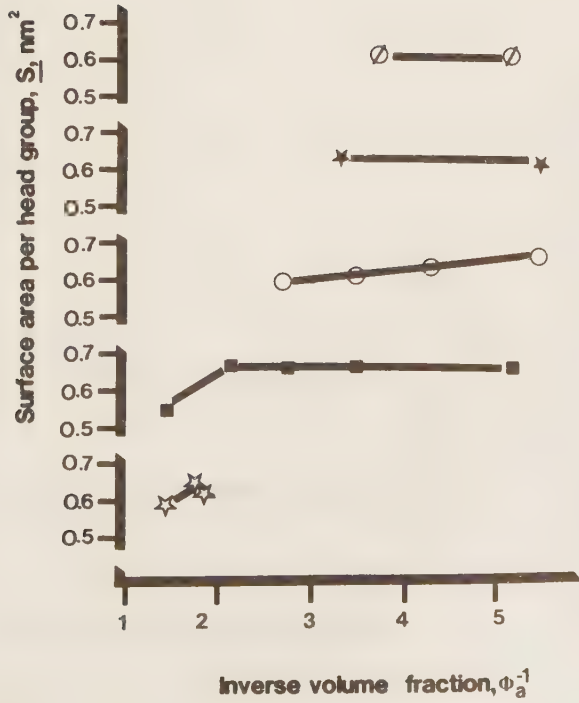


Figure 10.

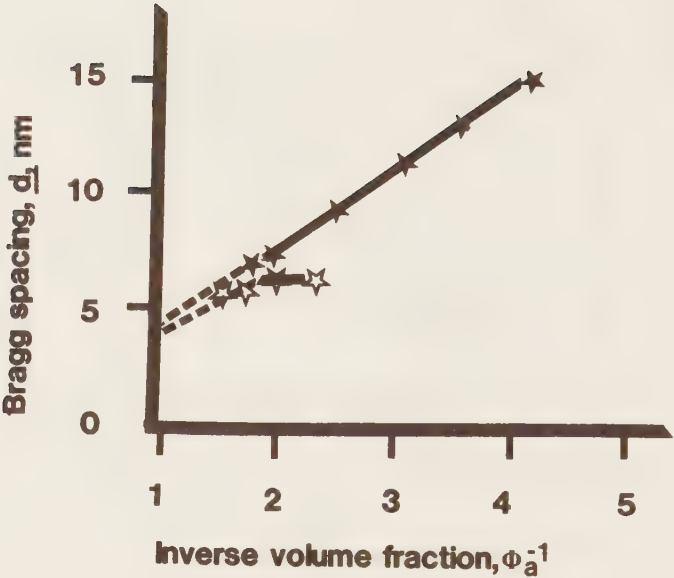


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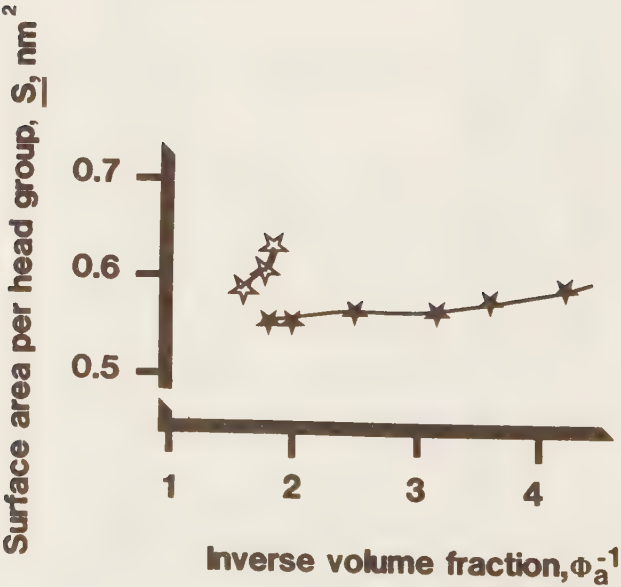


Figure 12.

TABLE I.

Thickness of lipid bilayer, d_a , in various environments extrapolated from d versus ϕ_a^{-1} plots. r = weight fraction of CTAB in CTAB + DMPC mixture

medium	r	d_a nm
water	0	4.0
"	0.01	3.6
"	0.02	3.8
"	0.03	3.7
"	0.058	3.3
"	0.10	4.1
Tris-HCl	0	4.2
buffer	0.026	4.0
pH = 7.4	0.051	4.9
10 mmole/dm ³		
NaCl	0	4.7
5 mmole/dm ³	0.05	4.1

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PAPER VI

**PHASE EQUILIBRIA AND VESICLE STABILITY
IN DIMYRISTOYL PHOSPHATIDYL
CHOLINE-CETYLTRIMETHYLAMMONIUM BROMIDE SYSTEMS**

Lisbeth Rydhag, Per Stenius and Lars Ödberg

**The Institute for Surface Chemistry,
Box 5607, S-114 86 Stockholm, Sweden**

Phase Equilibria and Vesicle Stability in Dimyristoyl Phosphatidylcholine-Cetyltrimethylammonium Bromide Systems

It is well known that liposomes or vesicles can be prepared from phospholipids and other dialkyl compounds which swell with water to form lamellar liquid crystalline phases. Recently, several methods have been described (1) which aim at the reproducible preparation of stable liposomes with a predictable size.

In our opinion, considerable insight into the conditions required for the formation of stable vesicles (or liposomes) may be obtained by considering the multi-component amphiphiles. As a model system we have studied the three-component system water/dimyristoyl-L- α -phosphatidylcholine (DMPC)/cetyltrimethylammonium bromide (CTAB) at 30°C. This temperature is above the chain melting temperature of DMPC (23.5°C, DTA analysis) and the Krafft point of CTAB (27°C). The DMPC contains >99.9% myristoyl chains and was free from polar impurities as checked by thin-layer chromatography.

Figure 1 shows the phase equilibria at very high concentrations of water. Pure DMPC (which is neutral between 3 < pH < 11 (2)) swells with water to a lamellar liquid crystal *D* (in Ekwall's nomenclature (6)) which incorporates a maximum 40% of water and is in equilibrium with an extremely dilute aqueous solution of DMPC ($\approx 10^{-10}$ mole/dm³ (3)). The swelling of the lamellar phase increases drastically if CTAB is added to the DMPC. As is seen in Fig. 1, CTAB may be incorporated up to a weight ratio $r = w(\text{CTAB})/[w(\text{CTAB}) + w(\text{DMPC})] \approx 0.5$. Higher ratios result first in a two-phase equilibrium between lamellar phase and an isotropic micellar aqueous solution of CTAB which solubilizes DMPC and then, for even higher r , in the formation of micellar solution only. For $r < 0.45$, the lamellar phase is in equilibrium with aqueous solution in which the concentration of CTAB is well below the CMC. Thus, in such equilibria the concentrations of the CTAB as well as of the DMPC is much higher in the lamellar phase despite the high solubility of the former compound in water.

We have studied the formation of vesicles by dispersing the lamellar phase into the aqueous solutions with which it is in equilibrium. We find that it is difficult to produce stable liposomes by dispersion of the DMPC/water phase without CTAB. Addition of a few mol% of CTAB, however, makes it very easy to produce stable and fairly monodisperse vesicles. The mean diameter of the vesicles which was deter-

mined from dynamic light scattering varied between 40 nm for vesicles with 1.9 mol% CTAB and 61 nm for vesicles with 8.93 mol% CTAB. The corresponding particle concentrations were 1.8×10^{14} and 7.7×10^{13} /dm³, respectively. It has been argued by Lansman and Haynes (4) that the interactions between dispersed vesicles can be understood on the basis of DLVO theory, i.e., they are colloidal stabilized by diffuse double layer repulsion. The rate constants for coagulation in the absence of repulsive diffuse double layer interactions, however, were found to be orders of magnitude smaller than predicted for diffusion-controlled coagulation. Details of their interpretation have been discussed by Nir and Bentz (5). Irrespective of the detailed picture of the interaction between liposomes in close contact with one another, it is obvious from our results that the effect of electrolytes on repulsive forces between disperse vesicles can be very clearly understood on the basis of the DLVO theory. We have studied the rate of coagulation by mono-, di-, and trivalent simple anions as a function of the concentration of CTAB in the vesicles in a stopped-flow spectrometer. The critical coagulation concentration (CCC) has been taken as the intersection on the log *W* versus log (electrolyte) concentration curve. The CCCs of the vesicles are given in Table I.

According to the DLVO theory, the CCC of two spheres with diameter *a* in a solution with dielectric constant ϵ_r is given by (7)

$$\text{CCC} = \frac{86\pi^2 R^3 T^5 k^3 \epsilon_0 \epsilon_r}{e^2} \cdot \frac{B^4}{A_H^2 z^6} = \text{const} \cdot \frac{B^4}{A_H^2 z^6} \quad [1]$$

where e_0 is the electronic charge, ϵ_0 is the permittivity of vacuum, A_H is the Hamaker constant, *z* is the counterion valency, and *R*, *T*, *k* have their usual meanings. At low surface potentials, $B \approx ze_0\phi_d/4kT$ and Eq. [1] may be written

$$\text{CCC} = \text{const} \cdot \frac{\phi_d^4}{A_H^2 z^2} \quad [2]$$

where ϕ_d is the Stern potential of the vesicles. At low potentials the relationship between the charge of the diffuse layer σ_d , the Debye length κ^{-1} of the diffuse layer and ϕ_d is

$$\sigma_d = \epsilon_0 \epsilon_r \kappa \phi_d = ze_0(2C_0\epsilon_0\epsilon_r/kT)^{1/2}\phi_d. \quad [3]$$

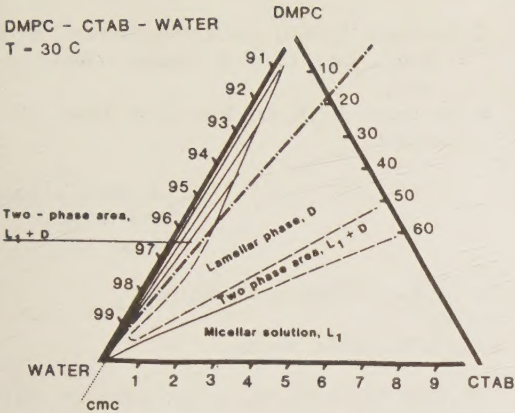


FIG. 1. Phase equilibria in the system H₂O/dimyristoylphosphatidylcholine/cetyltrimethylammonium bromide at 30°C. For CTAB/DMPC ratios higher than those indicated by the dotted line it was not possible to separate aqueous solutions from the lamellar phase by centrifugation. Concentrations are given in weight percent.

Introducing [3] into [2] we obtain

$$\log(\text{CCC}) = \frac{1}{3} \cdot \frac{\text{const}}{z^6} + \frac{4}{3} \log \sigma_d. \quad [4]$$

Thus, a plot of log(CCC) versus log σ_d should yield a straight line with slope 4/3.

We may now assume (a) that the charge on the vesicle surface is proportional to the concentration of CTAB in the vesicles: $\sigma_0 = \text{const} \cdot c(\text{CTAB})$, and (b) that the diffuse layer charge is proportional to σ_0 . Figure 2 shows a plot of log(CCC) for the vesicles against log $c(\text{CTAB})$. A straight line of slope 1.34 is obtained for coagulation with NaCl.

According to Eq. [2], the ratio between the CCC:s for $z = 1$, $z = 2$, and $z = 3$ at low potentials should be 1:0.25:0.11. For low CTAB concentrations, values in

TABLE I
Critical Coagulation Concentrations for DMPC/CTAB Vesicles

<i>C</i> _{CTAB}		CCC (NaCl) (mmole/dm ³)	CCC (Na ₂ SO ₄) (mmole/dm ³)	CCC (Na-citrate) (μ mole/dm ³)
Weight % in vesicles	Mole % in vesicles			
0.5	0.9	4		
1	1.9	13	3	
5	8.9	89		
10	17.2	500	14	0.1

Table I are in accordance with this prediction. For vesicles with 10% CTAB we actually find the ratio 1:0.028:0.002, which would be more in accordance with Eq. [1], which at high potentials (for which $B \rightarrow 1$) predicts the ratio 1:0.016:0.0014. However, it is well known that the dependency of the CCC:s on z often is much lower than expected from estimates of ϕ_d from measurements of, e.g., the ζ -potential (8). An explanation for this discrepancy is that the adsorption of counterions into the Stern layer is strongly dependent on z . It has been confirmed for vesicles of phosphatidylserine that the adsorption of counterions to the vesicle surface depends both on the valency (z) and the type of counterions (5).

If r is increased above ≈ 0.5 (the exact limit is very uncertain, since it is experimentally very difficult to establish the location of the phase boundary of the lamellar phase with very high contents of water) it becomes impossible to prepare stable liposomes. The lamellar phase for these high values of r is in equilibrium with micellar aqueous solutions. The high solubility of DMPC in the aqueous phase then would facilitate a rapid coalescence of the liposomes by diffusion.

We conclude that (a) the experimental conditions necessary to obtain stable liposomes from a mixture of charged and uncharged lipids can be precisely defined using knowledge about the phase equilibria for the mixture with water; and (b) the liposomes are electrostatically stabilized dispersions of liquid crystals in an aqueous solution with which the liquid crystal is in equilibrium.

Full experimental details and a more thorough discussion of the phase equilibria and vesicle stabilities will be published in forthcoming papers.

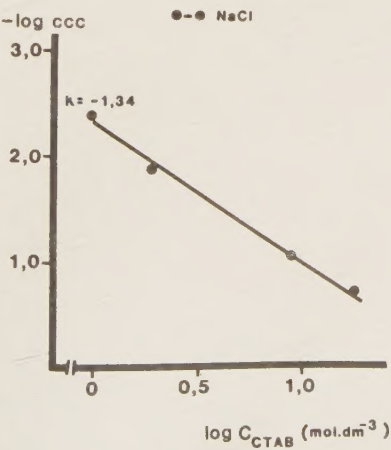


FIG. 2. Critical coagulation concentration for DMPC/CTAB vesicles as a function of the concentration of CTAB in the vesicles.

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LISBETH RYDHAG
PER STENIUS
LARS ÖDBERG

The Institute for Surface Chemistry
Box 5607
S-114 86 Stockholm, Sweden

Received March 26, 1981; accepted June 29, 1981

ISBN 91-7222-515-7
Sundt Offset Stockholm 82